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STUDIES TO CONTROL ENDEMIC TYPHOID FEVER IN CHILE

ANNUAL/FINAL REPORT

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A multi-faceted program of applied research has been undertaken in collaborati with the Ministry of Health of Chile intended to lead to control of erdemic typhoid fever in Santiago, Chile. These studies include: 1) Maintenance of prospective field trials evaluating the efficacy of Ty21a live oral typhoid vaccine given in various formulations and immunization schedules. 2) The firs evaluations of Ty21a vaccine in infants and pre-school children. 3) Develop- ment of a new enzyme-linked immunosorbent assay (ELISA) to measure Vi antibod and its use as a serologic screening test to identify chronic typhoid carriers

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4) Evaluation of a new oral antibiotic regimen to eradicate the chronic typho carrier state without resort to surgery. Keywords:

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SUMMARY

A multi-faceted program of applied research was undertaken in collaboration with the Ministry of Health of Chile intended to lead to control of endemic typhoid fever in Santiago, Chile. Information derived from these studies is directly applicable to the prevention of typhoid fever in United States military personnel deployed in endemic areas. During the life of the contract, activities that were emphasized included:

1) Studies of the epidemiology of endemic typhoid fever in Santiago, including descriptive epidemiological analyses, case/control studies, family-based studies, seroepidemiologic studies and studies to quantitate the occurrence of Salmonella typhi bacteremia in young children.

2) A quantitation of the magnitude of the reservoir of chronic S. typhi carriers in Santiago.

3) Evaluation of a serologic screening test to detect chronic S. typhi carriers in an endemic area (Santiago), based on measurement of passive hemagglutination antibody to highly purified Vi polysaccharide antigen.

4) Development of a new enzyme-linked immunosorbent assay (ELISA) to measure Vi antibodies capable of processing large numbers of sera and its use as a serologic screening test to identify chronic typhoid carriers.

5) Evaluation of oral antibiotic regimens to eradicate the chronic typhoid carrier state without resort to surgery.

6) Environmental bacteriologic studies to detect the presence of S. typhi in irrigation waters and other surface waters incriminated epidemiologically in the transmission of typhoid fever.

7) Clinical bacteriology studies comparing the sensitivity of blood, bone marrow and duodenal string cultures in the isolation of S. typhi from patients with suspect typhoid fever.

8) Molecular analyses of S. typhi strains from Chile for the presence of plasmids and examination of the electrophoretic patterns after cutting the plasmids with restriction endonucleases.

9) Initiation of four large-scale field trials of live oral typhoid vaccine Ty21a to assess the efficacy of various formulations and immunization schedules.

10) The first evaluations of Ty21a vaccine in infants and pre-school children.

FOREWORD

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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I. INTRODUCTION

Typhoid fever remains an important public health problem in many less-developed regions of the world and poses a risk for travelers from industrialized countries who visit such endemic regions. In virtually all endemic areas the incidence rates for typhoid fever are highest in children 5-19 years of age, i.e. school children. This is of potential relevance in the control of typhoid, since school children represent a "captive" population amenable to school-based immunization programs.

For United States military personnel who are stationed in less-developed areas or who must be prepared at short notice to operate in less-developed areas of geopolitical importance, typhoid fever represents an important potential health risk. The current vaccine utilized by the U.S. military forces to prevent typhoid fever, an acetone-inactivated preparation of whole Salmonella typhi inoculated parenterally, requires at least two doses given several weeks apart to immunize and causes high rates of significant adverse reactions. Therefore, a high priority has been given to identifying alternative typhoid vaccines that will provide significant protection without causing notable adverse reactions.

In areas where typhoid fever is endemic, the prevalence of chronic gallbladder carriers of S. typhi is often quite high. Thus, a particularly onerous risk of transmission of typhoid fever to U.S. military personnel in less-developed areas comes from foodhandlers from the indigenous population who may be chronic typhoid carriers and who unknowingly are involved in preparation of food. Under these circumstances, unwittingly, the potential exists for large epidemics to

occur. Furthermore, the size of the inocula of S. typhi present in food vehicles may be sufficiently high to overcome the protective efficacy of the current acetone-inactivated parenteral vaccine. Consequently, a simple, practical yet sensitive and specific screening test is required to screen large groups of individuals for the presence of suspected chronic typhoid carriers.

Dependents, including children, who accompany U.S. military personnel stationed on tours of duty in less-developed countries must also be protected against typhoid fever. In young children the subject of adverse reactions to the current parenteral typhoid vaccines is even more pertinent.

Since 1980, with support from the U.S. Army medical Research and Development Command, the World Health organization and the Pan American Health Organization, the Center for Vaccine Development of the University of Maryland has conducted an applied research program on the control of typhoid fever in Santiago, Chile, a highly endemic area. This applied research program has included epidemiological studies, environmental bacteriologic studies, comparison of methods for diagnosis of acute typhoid fever, development of new diagnostic and treatment methods for carriers, and large-scale field studies with Ty21a live oral typhoid vaccine. Results of these studies have direct relevance for the improved prevention of typhoid fever in U.S. military personnel.

Wherever possible, the results of the various components of the research carried out under this program will be provided in this FINAL REPORT by attaching scientific manuscripts that have been published or submitted.

II. EPIDEMIOLOGIC STUDIES OF ENDEMIC TYPHOID FEVER

A. Descriptive Studies

A detailed summary of the descriptive epidemiology of endemic typhoid fever in Chile, and in particular in Santiago, is contained in APPENDIX A.

B. Case/Control Study

A case/control study was carried out to identify risk factors, protective factors and vehicles of transmission. Results are contained in APPENDIX B. Prior to this study, it had been considered dogma among local epidemiologists that typhoid fever was transmitted within the home by food handlers (relatives or domestic servants) who were chronic typhoid carriers. The stool culture data obtained in this study were the first to demonstrate that it is rare to find chronic carriers among the domestic foodhandlers in homes of index cases of typhoid fever in Santiago. These observations implied that typhoid fever was contracted largely outside the home. Family studies were carried out to enlarge on these initial observations.

C. Family-Based Studies

Results of family-based epidemiological studies are contained in APPENDIX C. These studies corroborated that chronic carriers are rarely found in households of index cases of acute typhoid fever and showed that secondary transmission and concomitant cases within the households are rare.

D. Typhoid Fever in Infants

The peak age incidence of typhoid fever in Santiago, as in other endemic areas is in school age children, 5-19 years of age. In contrast, the reported incidence of typhoid fever is very low in infants and

toddlers. One of the possible explanations for this could be that young children less than two years of age do not consume the vehicles of transmission that are ingested by older individuals. However, it is also possible that contaminated vehicles are consumed by infants and toddlers but that these very young hosts do not clinically manifest an illness recognized as typhoid fever. A systematic collection of blood cultures was initiated in two primary health care clinics to answer this question. Results of this study are contained in APPENDIX D. It was found that bacteremia due to S. typhi and S. paratyphi occurred in approximately 4% of young children presenting to a primary health care facility with fever. In no instance was any child suspected clinically of having typhoid fever; therefore, this syndrome has been referred to as benign bacteremia due to S. typhi.

III. STUDIES OF CHRONIC TYPHOID CARRIERS

A. The Prevalence of Chronic Typhoid Carriers among Persons with Chronic Gallbladder Disease in Santiago, Chile.

Gallbladder contents were cultured from 1000 individuals undergoing cholecystectomy in seven hospitals in Santiago, Chile. Results are shown in APPENDIX E. Overall, S. typhi was recovered from 3.8% of the gallbladders.

B. A Precise Estimation of the Prevalence of Chronic Typhoid Carriers in Santiago

A precise estimate of the number of chronic biliary carriers of S. typhi was made using the detailed census of Santiago, data on the prevalence of gallbladder disease among individuals of various ages, and the measured prevalence of chronic carriage among persons with chronic gallbladder disease. Results are found in (APPENDIX F).

C. Serologic Screening to Detect Chronic Typhoid Carriers in an Endemic Area

A passive hemagglutination assay utilizing highly purified Vi antigen to measure Vi antibody was evaluated as a serological screening test to identify chronic typhoid carriers in a typhoid-endemic area, Santiago. The Vi serology proved to be very practical, sensitive and highly specific in identifying chronic carriers. The results are contained in APPENDIX G.

D. Development of an ELISA to Measure Vi Antibody and its Utility as a Serological Screening test for Chronic Typhoid Carriers

Based on the excellent results with the passive hemagglutination test for Vi antibody as a serological screening test to detect chronic typhoid carriers, an enzyme-linked immunosorbent assay (ELISA) was developed to measure Vi antibody. The ELISA utilized a tyraminated Vi polysaccharide as antigen. The advantages of the Vi ELISA include the ability to measure immunoglobulin class specific antibodies and the capacity to process very large numbers of sera. Results are shown in APPENDIX H.

E. Non-Surgical Domiciliary Treatment of Chronic Typhoid Carriers with a 28 Day Course of Amoxicillin and Probenecid

A 28 day oral regimen of amoxicillin and probenecid was evaluated as a non-surgical therapy to eradicate the chronic typhoid carrier state. A long-term cure was obtained in 15 of 26 carriers (56%). Those carriers who were successfully cured had a significantly higher serum antibiotic level than carriers in whom the treatment failed. Results are presented in detail in APPENDIX I.

F. Non-Surgical Antibiotic Therapy of the Chronic Typhoid Carrier State using Oral Ciprofloxacin

The new generation of quinolone antibiotics that has appeared in recent years includes ciprofloxacin, an agent with exceptionally good activity against S. typhi in vitro, with minimum inhibitory concentrations <0.06 mcg/ml. Pharmacokinetic studies in man indicate that the body fluid and tissue penetration of ciprofloxacin is excellent, including bile levels. For example, in a pilot study in which the bile levels of ciprofloxacin were measured after oral administration of 500 mg of ciprofloxacin, concentrations of drug of up to 10 mcg/ml were detected. Side effects of this antibiotic at either the 500 or 750 mg twice daily dosage schedule have been minimal. Based on these observations, we undertook a preliminary evaluation of ciprofloxacin in the treatment of chronic gallbladder carriers of S. typhi.

Twelve chronic carriers were enrolled into the study between June and December, 1985. Patients were treated with oral ciprofloxacin 750 mg twice daily, with careful monitoring for compliance and for possible adverse effects. Therapy was stopped in two cases after 10 days: one patient had an allergic reaction and one had a minimal drop in hematocrit of uncertain etiology. The remaining patients received the complete 28 day course of drug. Stool and bile-stained duodenal string cultures were obtained before therapy and at least monthly after discontinuation of therapy.

Of the total 12 carriers, one patient who completed the course of drug had a bacteriologic relapse within one week after completing therapy. A second patient whose stool and bile cultures were negative for six months following treatment became positive again for S. typhi. However, phage

typing of the isolates showed that the organism recovered after six months of negative cultures was distinct from the original infecting strain; thus this patient represents a re-infection. The other 10 patients have remained bacteriologically negative for at least six months, including the two individuals who had their courses of therapy interrupted before the full 28 days.

These preliminary results are extremely encouraging and suggest that ciprofloxacin is efficacious in treating chronic typhoid carriers and may achieve a higher cure rate than previous antibiotic regimens. Further, more comprehensive studies will undertaken to explore this possibility.

IV. ENVIRONMENTAL BACTERIOLOGY STUDIES

A. Recovery of S. typhi from Epidemiologically-Incriminated Surface Waters

Epidemiologic studies suggested that the lack of untreated sewage water for irrigation of salad vegetables during the dry summer months in Santiago represents a significant factor in the transmission of typhoid fever. Earlier environmental bacteriology studies, however, by Chilean bacteriologists had failed to recover S. typhi from the irrigation waters. Nevertheless, we proceeded to carry out environmental bacteriology studies using the same bacteriological methods as employed in the earlier studies but instituting the use of Moore swabs as the method of sampling the irrigation waters. By means of this new method of sampling, we were able to recover S. typhi repeatedly from surface waters used for irrigation. Details of these studies are contained in APPENDIX J.

B. Standardization of the Sensitivity of Moore Swabs for Isolating S. typhi from Environmental Sources

Moore swabs consist of large portions of gauze that are suspended for 48-72 hours in environmental sources to bacteriologically sample water; they act as filters to concentrate bacteria as the waters pass through the gauze. The sensitivity of Moore swabs in the recovery of S. typhi was evaluated by sampling sewers that drain the houses of known chronic typhoid carriers in Santiago. Results are presented in detail in APPENDIX K.

V. CLINICAL AND MOLECULAR BACTERIOLOGIC STUDIES OF S. TYPHI

A. Clinical Bacteriology Studies

The sensitivity of blood, bone marrow, and duodenal string cultures were compared in the isolation of S. typhi from 103 children with suspect typhoid fever. The combination of two blood and one duodenal string cultures equalled the sensitivity of a bone marrow in bacteriologically confirming the diagnosis of acute typhoid fever. These results are presented in detail in APPENDIX L.

B. Molecular Analyses of S. typhi

In the first study, 100 isolates of S. typhi from Santiago were examined for the presence of plasmids. Plasmids were found in only 8 isolates. None of the plasmids encoded antibiotic resistance. In fact, none of the 100 strains were found to be resistant to chloramphenicol, ampicillin or trimethoprim, the clinically important antibiotics in the treatment of typhoid fever. These results are contained in APPENDIX M.

In a second study, a total of 141 S. typhi strains, including 70 from Santiago and 71 from Lima, Peru were examined for the presence of plasmids. Plasmids were present in only 12 of 70 (17%) of the Chilean and

5 of 71 (7%) of the Peruvian strains. Identical 21 kilobase plasmids (as defined by restriction endonuclease digest patterns) were found in 13 of the 17 plasmid-containing S. typhi from Santiago and Lima. These results and their significance for epidemiologic studies are found in APPENDIX N.

VI. FIELD TRIALS OF EFFICACY OF LIVE ORAL TYPHOID VACCINE TY21a

A series of four separate field trials of efficacy have been carried out in Santiago, involving more than 640,000 schoolchildren. In these trials three different formulations of vaccine and several different immunization schedules were compared in randomized, controlled trials. The enteric-coated formulation was found to be significantly superior to a formulation consisting of gelatin capsules containing $NaHCO_3$ and lyophilized vaccine. Three doses of Ty21a in enteric-coated capsules given within one week has so far provided 67% efficacy for at least three years. Increasing the interval between doses to 21 days did not increase efficacy. Administering fewer doses (one or two) of vaccine in enteric-coated capsules provided lower levels of protection that endured for only two typhoid seasons. In contrast, administering four doses of enteric-coated vaccine conferred significantly higher protection than three doses. A fourth trial initiated in October, 1986, is comparing the relative efficacy of three doses of Ty21a given within one week in enteric-coated or liquid formulation. Results of these field trials of efficacy of Ty21a are contained in APPENDIX O and APPENDIX P.

VII. EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF A PURIFIED VI POLYSACCHARIDE PARENTERAL VACCINE

The safety and immunogenicity of a purified Vi parenteral vaccine was carried out in healthy young adults in Maryland and in Chilean Air Force recruits of the same age. Meningococcal polysaccharide served as the

control vaccine. Results are summarized in APPENDIX Q.

VIII. STUDIES WITH TY21a ORAL VACCINE IN INFANTS AND TODDLERS

A. Background

Live oral typhoid vaccine Ty21a has proven to be an important advance for the prevention and possible control of typhoid fever in endemic areas because it provides significant protection without causing adverse reactions. Although typhoid fever in endemic areas is largely a disease of schoolage children, the main delivery system for pediatric vaccines in most developing countries occurs through the expanded program on immunization (EPI) which is heretofore usually targeted exclusively at infants. Thus it is intriguing to consider whether immunization of infants might protect these children later when they reach schoolage. To even consider such a proposition it will be necessary to show that Ty21a is immunogenic in infants and young children. Because of the innocuity of Ty21a and the propensity of Salmonella to avidly interact with M cells of gut lymphoid tissue, many investigators have introduced genes encoding putative protective antigens of other organisms to obtain expression in Ty21a, thereby using the attenuated S. typhi as a "carrier" bacteria. Among the combinations reported so far are Ty21a expressing Shigella sonnei O antigen (1), B subunit of Escherichia coli heat-labile enterotoxin (LT) (2), and an outer membrane protein of Vibrio cholerae (3). Important target age groups for these bivalent vaccines are also infants and young children. Heretofore, however, the youngest age group to have received Ty21a vaccine is six year olds. We therefore initiated studies to evaluate the clinical acceptability and immunogenicity of Ty21a in infants and young children (less than five years of age) in Santiago, Chile, an area endemic for typhoid fever.

B. Materials and Methods

Vaccine was administered in three separate, randomized, placebo-controlled, double-blind studies.

1. Study #1

Study #1 involved healthy children 6-24 months of age recruited from the well baby clinic of the Centro Diagnostico of the Universidad Catolica School of Medicine, Santiago. Following explanation of the study to the parents and obtaining written consent, infants were randomized to receive three doses of vaccine (10^9 organisms per dose) or placebo which were given within eight days. Cups containing vaccine or placebo were prepared in a separate room by an unblinded nurse. She dissolved the contents of an enteric-coated capsule of Ty2la vaccine into 90 ml of cow's milk formula containing 0.5 gm of $NaHCO_3$. (A similar milk/bicarbonate "cocktail" method had been previously successfully used to vaccinate Chilean six year olds who demonstrated a good serologic response post-vaccination) (20). Milk containing $NaHCO_3$ alone served as the placebo. The coded cups containing vaccine or placebo were presented to a second nurse who administered the contents to the children in double blind fashion. The infants were examined 24 and 48 hrs after each dose of vaccine at which time the child's temperature was recorded; axillary temperatures were obtained because this is the accepted custom in Chile. The mother was interviewed to elicit evidence of adverse reactions in the previous 24 hrs.

A 4 ml specimen of blood was collected prior to and 21 days after vaccination. The blood was passed through a Ficoll-Hypaque column to obtain mononuclear cells to carry out lymphocyte replication studies with selected S. typhi and appropriate control antigens to measure the

cell-mediated immune response to vaccination with Ty21a. Plasma was utilized to measure serum antibodies: IgG antibody to O antigen was measured by enzyme-linked immunosorbent assay (4); H antibody was measured by Widal tube agglutination as previously described (5) and Vi antibody was detected by passive hemagglutination using highly purified Vi polysaccharide (5).

2. Study #2

This study was carried out among children 2-5 years of age (three-fourths were three or four year olds) in a nursery school in the Pincoya district of Area Norte, Santiago. Children of consenting parents were randomized to receive three doses of Ty21a vaccine (10^9 viable organisms per dose) or placebo given within a period of eight days. Capsules of vaccine were opened by an unblinded individual in a separate room and the contents suspended in 50 ml of cow's milk containing 0.75 gm of $NaHCO_3$; placebo consisted of milk with bicarbonate only. The coded cups containing vaccine or placebo were presented to a nurse who distributed them to the children in double blind fashion. Children were examined 24 and 48 hrs after vaccination at which time axillary temperatures were taken and the parents were interviewed.

Before vaccination and 21 days thereafter 4 ml of blood were collected and the sera separated and frozen to be tested later for antibody as described above.

3. Study # 3

The third study was carried out in 2-5 year old children in a second nursery school in Pincoya where children of consenting parents were randomized to receive four doses of Ty21a vaccine (10^9 viable organisms per dose) or placebo. Vaccine was administered identically as in Study # 2 but a fourth dose was given within the eight day period in attempt to

increase vaccine immunogenicity. Blood was collected before and 21 days after vaccination for serologic tests as described above.

C. Results

1. Clinical Response to Vaccine

Table 1 shows the number of children in each study who received vaccine or placebo and the frequency of adverse reactions. Diarrhea, fever, vomiting and abdominal pain were uncommon in either group with no difference evident between vaccine and placebo recipients.

2. Immune Response to Ty21a

The serologic response to vaccination of infants and young children is summarized in Table 2. In Study #1, involving infants and toddlers less than two years of age, no significant rises in O antibody measured by IgG-ELISA were detected. Because these results contrast so notably from the serologic response of six year olds administered Ty21a vaccine by this method of administration in a previous study (4), we proceeded in the next study to vaccinate slightly older children, 2-5 years of age. These children in Study # 2 showed some serologic reactions to both O and H antigens; in total 8 of 24 vaccinees showed a significant rise in one or another serologic test versus 0 of 25 pre-school children who received placebo ($p<0.002$).

In the third study, we attempted to increase the immunogenicity of the vaccine by administering an additional dose to preschool children. The addition of a fourth dose did not increase the serologic response to the vaccine.

Ty21a vaccine does not contain Vi antigen and therefore even in older individuals does not stimulate Vi antibodies. Thus the total lack of serologic response to Vi where measured in these studies in young children

is completely as expected (Table 2).

Lymphocyte cultures from the vaccinated and placebo infants responded to mitogens. However, the lymphocytes collected post-vaccination failed to show evidence of replication in the presence of S. typhi O polysaccharide or control (S. thompson or E. coli) O polysaccharides.

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TABLE 1

OCCURRENCE OF ADVERSE REACTIONS IN INFANTS
AND PRE-SCHOOL CHILDREN FOLLOWING INGESTION
OF LIVE ORAL TYPHOID VACCINE TY21A VACCINE OR PLACEBO

Study	Age Group	No. Doses	Diarrhea		Fever		Vomiting		Abdominal Pain	
			Vaccine	Placebo	Vaccine	Placebo	Vaccine	Placebo	Vaccine	Placebo
TyCh 6001	6-24 mos.	3	2/18*	1/19	2/18	2/19	3/18	2/19	-	-
TyCh 6003	2-5 yrs.	3	0/24	0/25	0/24	0/25	1/24	0/25	2/24	0/25
TyCh 6004	2-5 yrs.	4	1/17	2/24	1/17	0/24	0/17	1/24	1/17	2/24
Totals			3/59 (5.1%)	3/68 (4.4%)	3/59 (5.1%)	2/68 (2.9%)	4/59 (6.8%)	3/68 (4.4%)		

* No. positive/No. vaccinated

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TABLE 2

SEROLOGIC RESPONSE FOLLOWING VACCINATION OF INFANTS AND YOUNG CHILDREN WITH LIVE ORAL VACCINE TY21A OR PLACEBO

Study	Age Group	No. Doses	O Antibody by IgG-ELISA		H Antibody by Widal		Vi Antibody by PHA[$]		Rises by Any Assay	
			V[*]	P[*]	V	P	V	P	V	P
TyCh 6001	6-24 mos.	3	0/18[+]	0/19	0/18	0/19	0/18	0/19	0/18	0/19
TyCh 6003	3-5 yrs.	3	5/24	0/25	3/24	0/25	0/24	0/25	8/24	0/25
TyCh 6004	3-5 yrs.	4	0/17	1/24	0/17	1/24	NT	NT	0/17	1/24
Totals			5/59	1/68	3/59	1/68	0/42	0/44	8/59	1/68

[*] V = vaccine group, P = placebo group

[+] No. positive/No. vaccinated

[$] Positive hemagglutination using highly purified Vi antigen

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INTERVENTIONS TO CONTROL ENDEMIC TYPHOID FEVER: FIELD STUDIES IN SANTIAGO, CHILE[1]

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Introduction

Typhoid fever, the acute, often debilitating, febrile illness representing generalized infection of the reticuloendothelial system, intestinal lymphoid tissue and gall bladder, is endemic in many less-developed areas of the world. Man is the sole reservoir and host of the infection (Figure 1), in contrast with other *Salmonella*, which are typically zoonotic infections of domestic and herd animals (*1*). Approximately 3-5% of acute *S. typhi* infections result in chronic gall bladder infection, giving rise to long-term biliary carriers. The propensity to become a carrier following acute infection increases with age and is greater in females, thus paralleling the epidemiology of gall bladder disease (*2–4*). Asymptomatic chronic carriers comprise the reservoir that maintains the endemicity of the disease by contamination of food and water vehicles (Figure 1); direct contact spread of typhoid fever is relatively uncommon (*1*).

Recognition of the above-mentioned facts helps explain most of the observations regarding the global occurrence of typhoid fever. It is endemic in less-developed areas where sanitation and food hygiene are primitive. However, the highest incidences occur where piped water is available but the water is fecally contaminated and untreated, a situation prevalent in many large cities of Europe and North America in the late 19th century (*5–7*). This phenomenon of piped transport of impure water can be regarded epidemiologically as an example of amplification of disease transmission.

The introduction of purification (including chlorination) of water supplies and treatment of sewage prior to discharge, interrupted the amplification step and caused a precipitous fall in the incidence of typhoid fever in the cities of Europe and North America in the first three decades of the 20th century (*5–7*). Figure 2 illustrates this drop in incidence of typhoid fever in the United States. This pattern is typical of virtually all countries as they industrialize and provide chlorinated water and sewerage systems for their urban populations (*8*).

One country, Chile, in the cone of South America, represents an exception to the above pattern. By most criteria of health and quality of life, Chile is advanced well beyond the

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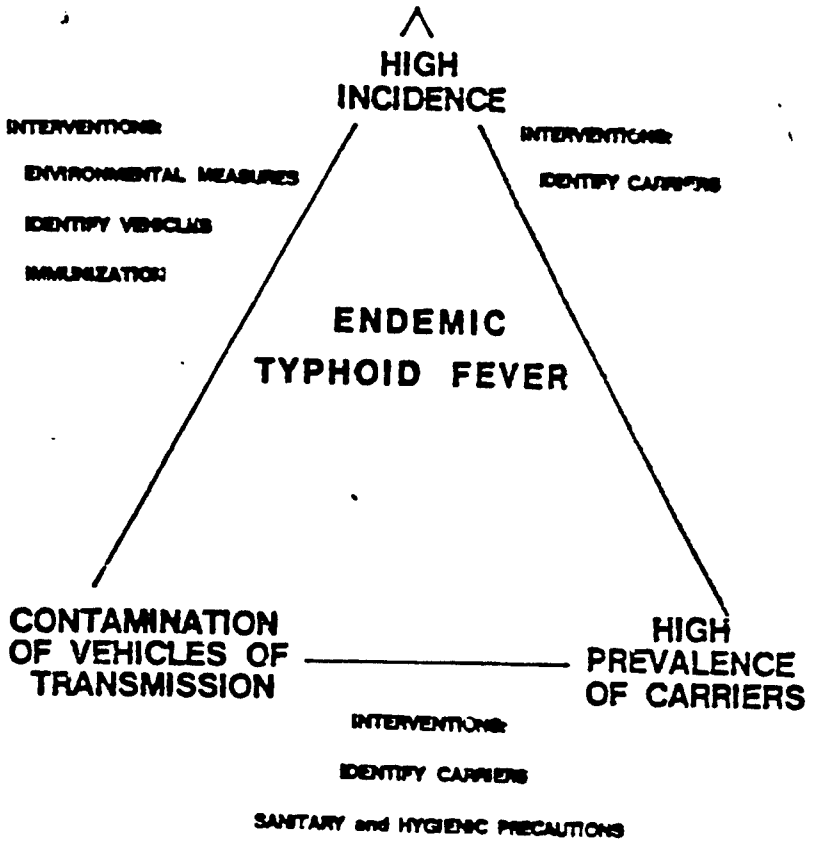
Figure 1. A schematic diagram of the cycle of endemic typhoid fever and intervention points.

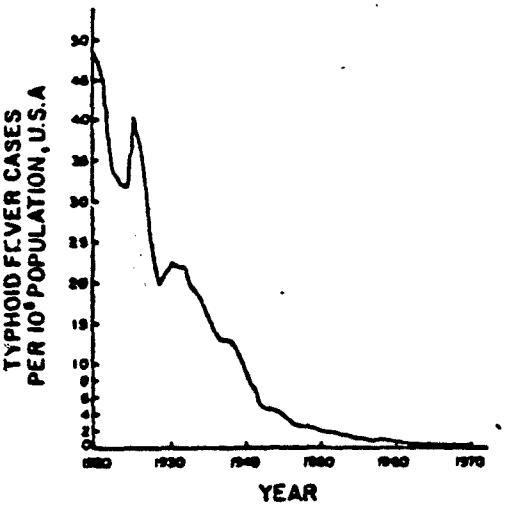


Chronic carriers comprising the reservoir contaminate food and water vehicles. These are consumed by susceptible hosts leading to a high incidence of typhoid fever. Approximately 3-5% of infected persons become chronic gall bladder carriers of *Salmonella typhi* and perpetuate the reservoir. The various interventions are discussed in the text.

ranks of the less-developed countries. Chile has a world-renowned health care delivery system, a low infant mortality rate (Table 1), most immunizable communicable diseases have been impressively controlled (Table 1), and the literacy rate is 94%. In the capital city, Santiago, 96% of households have bacteriologically monitored, chlorinated water and 75% have connections to the municipal sewerage system (9). Nevertheless, despite these manifestations of a high level of development and control of most other communicable diseases, typhoid fever is highly endemic in Chile, particularly in Santiago. The incidence of typhoid fever in Chile from 1960 to 1981 is shown in Table 2. During that period, the incidence rate has usually exceeded 50 cases per 10^5 population; since 1977 the rate has surpassed 90 per 10^5 in Chile and 150 per 10^5 in Santiago.

Santiago, Chile thus provided a unique opportunity to intensively study the epidemiology of endemic typhoid fever and its control in a country of relatively advanced technology and educational development. Accordingly, following some preliminary epidemiological

Figure 2. The incidence of typhoid fever per 100,000 population in the United States from 1920 to 1970.



and seroepidemiological studies in late 1978, a multifaceted program "Studies to Control Endemic Typhoid Fever in Chile" was designed. The ultimate goal of the program is to reduce the incidence of typhoid fever in Chile to the level where it no longer represents a major public health burden. The specific objectives of the program have included (Figure 1):

1) Epidemiological investigations to identify high-risk groups, risk factors, protective factors, major modes of transmission and specific contaminated food and water vehicles.

2) A quantification of the magnitude of the reservoir of chronic *S. typhi* carriers.

3) Development of a simplified, sensitive, and specific serological assay to screen large numbers of food handlers and other epidemiologically important groups for the presence of chronic typhoid carriers.

4) Evaluations of possible nonsurgical domiciliary antibiotic treatments to eradicate the chronic carrier state.

5) Environmental bacteriology studies to confirm the presence of *S. typhi* in epidemiologically incriminated waters.

6) Large-scale controlled field trials to assess the efficacy of a live oral attenuated *S. typhi* vaccine (strain Ty21a) in the prevention of typhoid fever in Chilean schoolchildren and its use as a public health intervention.

Each of these components of the program will be reviewed below. The project represents a collaborative effort involving participants from several institutions in Chile and several abroad as well as international agencies. Included are the Chilean Ministry of Health, the Center for Vaccine Development of the University of Maryland School of Medicine, the Pan American Health Organization, the World Health Organization, the Walter Reed Army Institute of Research and the Swiss Serum and Vaccine Institute.

Epidemiological Investigations

Descriptive Epidemiology

Chile is a long narrow country stretching more than 3,000 miles from north to south. Approximately 4.5 million of Chile's 11.8 mil-

Table 1. Infant mortality rate and incidence of certain immunizable communicable diseases in Chile, 1964–1980.

Year	Measles		Pertussis		Poliomyelitis		Infant Mortality Rate
	Cases	Incidence	Cases	Incidence	Cases	Incidence	
1964	35,941	428.3[a]	5,279	62.9[a]	363	4.3[a]	105.3[b]
1969	9,538	99.7	2,905	30.4	64	0.7	78.7
1975	8,413	82.1	2,550	24.9	2	0.0	55.4
1980	3,844	34.0	2,795	25.2	0	0	31.8

[a] Rate per 100,000.
[b] Rate per 1,000 live births.

Table 2. Population size, number of cases of typhoid fever, and morbidity rates for typhoid fever in Chile and Metropolitan Santiago, 1960–1981.

	Chile			Santiago		
Year	Population	No. of cases	Rate per 10^5	Population	No. of cases	Rate per 10^5
1960	7,585,350	4548	59.6	2,439,093	2,078	85.2
1961	7,770,270	4618	59.2	2,530,593	2,401	94.9
1962	7,955,190	3873	48.9	2,622,094	2,034	77.6
1963	8,140,110	4185	50.9	2,713,595	2,158	79.5
1964	8,325,030	4732	56.0	2,805,096	2,731	97.4
1965	8,509,950	5596	64.8	2,896,596	2,754	95.1
1966	8,681,671	4576	51.5	2,984,350	2,688	90.1
1967	8,853,393	4536	49.5	3,072,103	2,747	89.4
1968	9,025,115	7091	75.8	3,159,857	4,590	145.3
1969	9,196,837	5358	46.0	3,247,610	3,463	106.6
1970	9,368,558	5344	57.0	3,334,936	3,408	102.2
1971	9,545,449	4784	50.1	3,425,061	3,007	87.8
1972	9,722,341	4527	46.6	3,514,220	2,640	75.1
1973	9,899,231	3656	37.3	3,603,465	1,865	51.8
1974	10,076,123	4655	46.2	3,693,767	2,424	65.6
1975	10,253,014	6110	59.6	3,786,016	3,500	92.4
1976	10,454,387	6150	59.1	3,879,626	3,545	91.4
1977	10,655,757	11,533	108.2	3,974,437	7,070	177.9
1978	10,857,128	13,114	120.8	4,070,293	8,334	204.8
1979	11,058,498	10,760	97.3	4,167,000	6,356	152.6
1980	11,259,871	10,872	96.6	4,264,518	6,827	160.1
1981	11,477,150	10,759	94.0	4,363,026	6,936	159.0

lion inhabitants live in metropolitan Santiago, which is located in the center of the country in a valley between the Andes mountains and the Pacific Ocean. Santiago has a temperate "Mediterranean" climate with wet winters and rainless summers.

In Table 2 are listed the population of Santiago and of Chile, the cases of typhoid fever, and incidence rates from 1960–1981. Approximately one-half the cases of typhoid fever in any year are reported from Santiago. In 1977 the incidence of typhoid fever doubled and remained at elevated rates for several years. It is not clear what factors were responsible for the doubling of the notification rates for typhoid fever since 1977. It is apparently not due to an administrative change in notification, since there was not a similar rise in nonenteric infections.

Typhoid fever shows a striking seasonality in Santiago, where it is a warm-season disease. Approximately 65% of cases occur between December 1 and April 30 of each y (Table 3).

The incidence of typhoid fever drops as goes further north or south from Santia One area of particular interest is the Los gos Region, where many persons from S tiago vacation in summer. The daytime te peratures in this region can be quite warm summer, but there is rainfall all year lo Typhoid is very uncommon here.

Table 4 shows the incidence of typhoid ver by administrative area of Santiago in years 1977 and 1978. Area Oriente, an are affluence, has high rates in addition to poorer areas of Santiago. Furthermore, incidence of typhoid in Area Oriente is lieved to be very underreported because m febrile children there are cared for by pri pediatricians who do not readily notify ca to the Ministry of Health. In contrast,

Table 3. Mean number of typhoid fever cases by month in Santiago, Chile, 1970–1976 and 1977–1981.

Month	Mean No. of cases 1970–1976	% of Total	Mean No. of cases 1977–1981	% of Total
January	421	14.1	948	13.3
February	403	13.5	917	12.9
March	415	13.9	1015	14.3
April	400	13.4	867	12.2
May	302	10.1	605	8.5
June	183	6.1	529	7.4
July	103	3.5	282	4.0
August	76	2.5	124	1.7
September	62	2.1	162	2.3
October	109	3.7	215	3.0
November	167	5.6	601	8.5
December	340	11.4	841	11.8

Table 4. Incidence rates of typhoid fever by administrative area, Santiago, Chile.

Area	Mean socioeconomic level	Incidence rate/10^5 1977	1978
Sur	Low, middle	317.5	321.5
Sur Oriente	Low, middle	117.0	113.1
Occidente	Low, middle	140.9	177.0
Norte	Low, middle	125.5	220.7
Central	Middle	185.3	168.6
Oriente	Middle, upper middle, high	118.7	161.9

proximately 80–90% of children in the other areas of Santiago are cared for by physicians at National Health Service facilities where reporting is compulsory.

The highest incidence of typhoid fever is found in 10–14 year olds (Table 5); approximately 60% of cases occur in school-age children 6–19 years of age. In contrast, notification rates in children less than 2 years of age are very low. Notable sharp increases in the incidence in childhood occur in 3 year olds (versus 2 year olds) and in 6 year olds (versus 5 year olds).

In summary, an explanation for the endemicity of typhoid in Santiago must explain:

- the high incidence in schoolchildren.
- a summer seasonality with the highest incidence (December to mid-March) occurring when children are out of school on summer recess.
- an apparent low incidence in children less than 2 years of age.
- amplified transmission despite high levels of sanitation.
- why the incidence of typhoid fever is very low in the Los Lagos Region, despite the influx into that region of many persons (including presumably chronic carriers) from Santiago.

Seroepidemiology

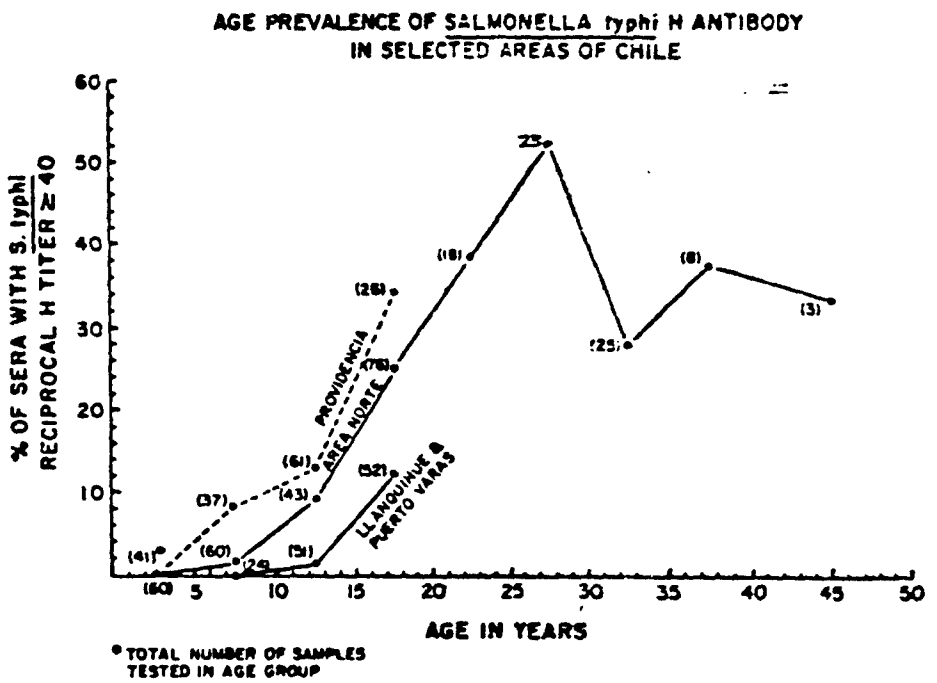
Antibodies to the flagellar (H) antigen of *S. typhi* (d) are IgG and long-lived (*10*). They may derive from immunization or clinical or subclinical infection (*10*). Where parenteral vaccine is not commonly used, as in Chile, measurement of the prevalence of *S. typhi* H antibodies can give helpful insights into the epidemiology of typhoid fever (*10*). In November 1978, sera were obtained from healthy 15–19 year olds, as well as other age groups, in three areas of Chile. These included: 1) Area Norte, Santiago, representing a low and low-middle socioeconomic population; 2) children from an exclusive private school in Providencia, Area Oriente, representing an affluent group; and 3) schoolchildren in the Los Lagos Region of southern Chile, where the reported incidence of typhoid fever is low. As shown in Figure 3, 25% of 15–19 year olds in Area Norte had *S. typhi* H agglutinins at a reciprocal titer of 40. The prevalence in Providencia 15–19 year olds was as high (in fact slightly higher, 34%). In contrast, the prevalence of antibodies in teenagers in the Los Lagos Region (12%) was significantly lower than in Santiago ($p=0.04$). The seroepidemiological data confirm the notification data regarding the occurrence of typhoid, i.e., it is indeed common in high socioeconomic areas of Santiago but is rare in the Los Lagos Region.

Table 5. Age-specific incidence rates and cases of typhoid fever in Santiago, Chile, 1970–1976 and 1977–1981.

	1970–1976			1977–1981			
Age group	Mean No. cases	% of Total	Mean incidence per 10^5	Mean No. cases	% of Total	Mean incidence per 10^5	Increase[a]
0–4	170	5.9	38.7	421	6.4	89.2	2.3
5–9	524	18.3	126.5	1193	17.1	272.2	2.2
10–14	624	21.8	152.2	1413	20.3	333.0	2.2
15–19	497	17.3	135.5	1465	21.0	283.4	2.1
20–24	421	14.7	126.4	728	10.5	246.7	2.0
25–34	407	14.2	72.0	1023	14.7	153.3	2.1
35–44	134	4.7	33.6	366	5.3	74.6	2.2
45–54	52	1.8	17.4	179	2.6	50.2	2.9
55–64	23	0.8	10.8	86	1.2	36.0	3.3
65	9	0.3	5.2	79	1.1	38.5	7.4

[a] 1977–1981 rate over 1970–1976 rate.

Figure 3. The prevalence of *Salmonella typhi* H antibodies in various age groups from three population groups in Chile.



1) Area Norte, a low and middle socioeconomic section of Santiago with a high reported incidence of typhoid fever; 2) children from a private school in Providencia, an affluent area where, nonetheless, the incidence of typhoid fever is high; 3) Llanquihue and Puerto Varas, in the Los Lagos Region of Southern Chile where the reported incidence of typhoid fever is low.

Case/Control Study

A case/control study attempted to identify specific vehicles of transmission as well as risk factors and protective factors (*11*). This study, which involved 81 cases age 3–14 years and 81 matched controls, incriminated only one possible vehicle, flavored ices sold by street vendors. One aspect of the study involved the collection of multiple coprocultures from the food handlers in both the case and control households. Chronic *S. typhi* carriers were identified in only 2 of the 81 (2.5%) cases and 1 of 81 (1.2%) control households. This observation was the first evidence to demonstrate that chronic typhoid carriers in the home are not responsible for most cases of typhoid fever in children in Santiago.

Family Studies

We sought to further examine factors involved in the transmission of *S. typhi* in Santiago by interviewing and culturing the household members of recently confirmed pediatric cases. This represents a more intensive study of the household as the possible site in which transmission of typhoid infection may be occurring. Two separate studies involving 24 and 39 households, respectively, were carried out in which attempts were made to identify chronic typhoid carriers as well as possible concurrent (or secondary) cases by culturing household foodhandlers and contacts below 19 years of age (these represent high-risk individuals) (*12*, C. Ferreccio *et al.* unpublished data).

Ninety-six percent of the households had municipal water and 79% were connected to the city sewerage system. A chronic *S. typhi* carrier was identified in only one (1.6%) household. Most importantly, only two concomitant cases were identified among the scores of high-risk children less than 19 years of age who were cultured. Eighty-six percent of index cases gave a history of eating outside the household at least once each week.

In summary, the data from family-based studies confirm the earlier observation that it is uncommon to find a chronic carrier in the household of an index case. Furthermore, the low frequency of concomitant cases among high-risk siblings strongly suggests that the vehicle of transmission in children and teenagers is usually consumed outside the home, otherwise more concomitant cases would be expected.

S. typhi Infection in Infants

Few cases of typhoid fever are reported in children less than 2 years of age in Santiago. This could represent a lack of consumption of the vehicles that transmit *S. typhi* to older children or could imply that, following ingestion of the usual vehicles of transmission, infants manifest an atypical response to infection that is not readily recognized clinically. To help resolve this question we systematically performed blood cultures in children less than 2 years of age with fever who were seen at two health centers in Santiago during the three peak months of the typhoid fever season (*13*). Of 197 outpatients less than 24 months of age with fever who were cultured, *S. typhi* was isolated from the blood cultures of four infants (2%), *S. paratyphi* B from two (1%) and *S. paratyphi* A from one (0.5%). The clinical syndrome in these infants was very mild, consisting of 1–5 days of fever (38.3–38.8°C) and respiratory symptoms. In no instance was enteric fever considered in the differential diagnosis and, were it not for the study protocol, a blood culture would not have been taken from any infant.

These data demonstrate that during the typhoid fever season in Chile children less than 2 years of age are becoming infected at a much higher rate than previously appreciated. The mode of transmission and specific vehicles have yet to be identified.

Environmental Bacteriology Studies

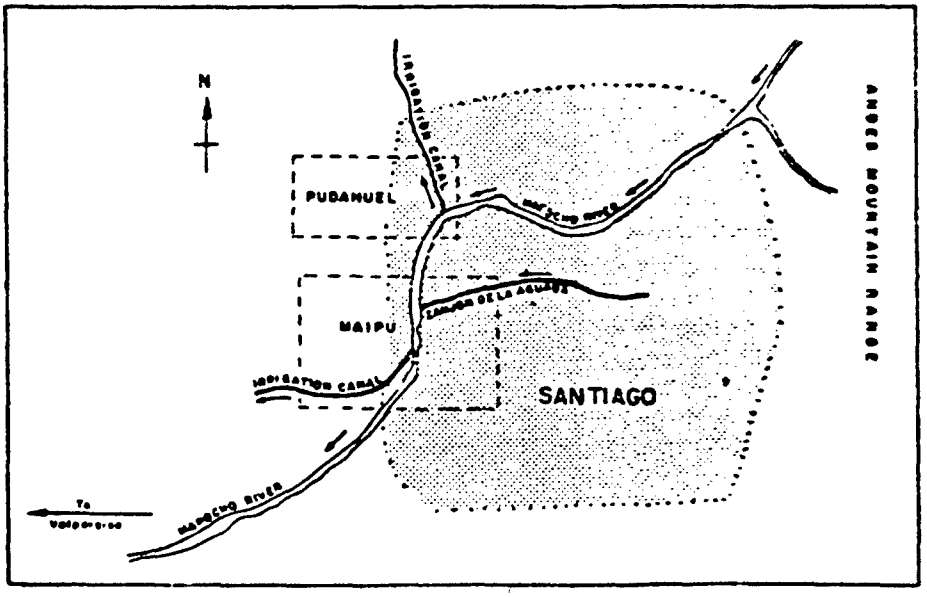
The observation that most cases of typhoid fever are not associated with a chronic carrier in the home made us turn our attention to a closer inspection of the water and sewerage system. We learned that although three-fourths of Santiago households were connected to the sewerage system there is no treatment of sewage. Thus, raw, untreated sewage enters the Mapocho River (which traverses northern Santiago) or the Zanjón de la Aguada (a large open sewer that traverses southern Santiago from east to west before emptying into the Mapocho River southwest of the city) (Figure 4). As the Zanjón and the Mapocho River reach the westernmost portion of metropolitan Santiago, their fecally polluted, untreated waters are diverted for irrigation of crops during the rainless summer season. Prior to May 1983, 90% of the crops grown in this region were lettuce, cabbage and celery, vegetables that are difficult to wash and are eaten raw in salads in Chile.

The hypothesis that raw vegetables grown with untreated waste waters and fruit "freshened" with contaminated river water represent important vehicles of transmission successfully explains the following epidemiological observations.

- the striking seasonality of typhoid (irrigation is used in the summer when there are no rains).
- the low reported incidence in young children (raw vegetables are not an important food item for infants and toddlers).
- the high incidence of typhoid fever in high socioeconomic neighborhoods of Santiago (they eat salads in restaurants and at home).
- the low incidence of typhoid fever in the Los Lagos Region of Chile (because of year-round rains in this region, irrigation is not used).

Prior to 1983, Chilean microbiologists carried out a series of environmental microbiology studies in attempts to isolate *S. typhi*

Figure 4. A map showing the two major water channels, the Mapocho River and the Zanjón de la Aguada, which receive untreated sewage as they traverse Santiago.[a]



[a] These untreated wastewaters are used for irrigation of vegetables in Maipú and Pudahuel during the rainless summers.

from waters of the Zanjón and the Mapocho River and from vegetables irrigated with untreated wastewater (*14–16*). While heavy coliform counts and many nontyphoidal *Salmonella* were found, *S. typhi* was never isolated from vegetables or from the Zanjón and was isolated only once from the Mapocho River (*17*). The past failure to isolate *S. typhi* from the polluted waters was in conflict with our epidemiological incrimination of this wastewater. In review of the earlier Chilean studies, we concluded that the bacteriological methods were efficient, but the techniques of environmental sampling appeared suboptimal. Therefore we initiated new environmental bacteriological studies (*18*) using Moore swabs (*19–21*) (thick wads of cotton gauze which are left in the flowing wastewater for 2–3 days allowing the gauze to act as a filter) to collect samples. The Moore swab, originally described in England in 1948 (*19*), is a concentrating method that has been used successfully to locate the homes of chronic *S. typhi* carriers by isolating the organism from sewage effluents (*19–24*). Moore swabs have been extremely useful in the investigation of urban typhoid fever outbreaks in Europe, Japan, and the United States of America (*19–24*). However, the efficacy and reliability of Moore swabs in endemic areas had not previously been assessed. Nevertheless, based on its success in finding *S. typhi* in sewage contaminated by carriers in industrialized areas, we decided to employ modified Moore swabs to isolated *S. typhi* from environmental sources in Santiago.

Microbiological examination of rivers and irrigation canals of Santiago, Chile was carried out from January to March 1983. The two major waterways in Santiago that carry wastewater are the Mapocho River in the north and the Zanjón de la Aguada canal in the south (Figure 4). Untreated sewage flows directly into these waters, which are used for irrigation in the agricultural districts of Maipú and Pudahuel (on the perimeter of the city). The Zanjón de la Aguada, which is heavily contaminated with industrial waste from the central section of the city, receives untreated sewage and becomes fecally polluted as it flows westward. During the final few kilometers as it approaches the agricultural areas, no further sewage is discharged in an attempt to allow a degree of self-purification of the wastewater.

We placed 133 swabs into the Mapocho River and the Zanjón de la Aguada and recovered 93. None of the 17 swabs placed in industrial areas grew *S. typhi*. In contrast, 4 of the 31 swabs from the Zanjón de la Aguada without industrial discharge (13%) and 4 of 45 from the Mapocho River (8.3%) contained *S. typhi*. Of the 76 swabs placed in agricultural areas, 8 were culture-positive (11%). Of the 8 isolates, 5 were phage type E1 and 46, the two most common disease-causing types in Chile, one strain was untypable, and the other two were N and M1.

Thus, using Moore swabs, we were able to isolate *S. typhi* from irrigation water in Santiago, Chile. Since the sensitivity of the Moore swab is inversely related to the size of the waterway sampled (*21*), our isolation rate of 11% from these large waterways is probably an underestimate. *S. typhi* is fastidious, easily inhibited by coliforms, and usually present in relatively small numbers in environmental samples (*22*). The Moore swab, by acting as a filter, improves the chance of isolating rare *S. typhi* among millions of coliforms, and we have now shown it to be a practical, reliable tool to isolate *S. typhi* from irrigation water in endemic areas. Finding *S. typhi* with the same phage types as disease-causing isolates in irrigation water supports the hypothesis, based on epidemiological observations, that contaminated vegetables in Santiago serve as important vehicles of transmission.

Studies with Chronic Carriers

Quantitation of the Reservoir

Using epidemiological techniques, we estimated that in 1980 there existed 25,019 fe-

male and 4,575 male chronic *S. typhi* carriers among the 4,264,515 inhabitants of Santiago, giving a prevalence of 694 carriers per 10^5 population (*24*, *25*).

Simple Serological Screening Test for Carriers

Simple, yet reliable, screening tests are required to allow rapid and effective identification of chronic carriers. Shortly after the original description of the Vi antigen by Felix and Pitt in the 1930s (*26*), they noted that chronic carriers had high titers of Vi antibody (measured by bacterial agglutination using Vi-rich bacteria) and suggested that this serology might serve as a screening test to detect carriers (*27*). Over the next 45 years, great debate occurred on this subject due to widely divergent results of various investigators (*28–31*). Until recently, all assays were limited by the lack of purity of the antigen. However, a few years ago highly purified Vi antigen became available (*32*). Vi serology using this purified antigen in a passive hemagglutination (HA) test was successful in detecting chronic carriers in outbreak situations in nonendemic areas (*33*). Therefore we undertook to evaluate the utility of this serological screening test to identify carriers in an endemic area, Santiago, Chile (*34*). Sera were tested from the following Chilean populations:

1) 36 bacteriologically-confirmed known chronic carriers.
2) 29 patients of both sexes, age 18 years and over, with acute typhoid fever.
3) 388 women who had confirmed typhoid fever 12–48 months earlier and who were apparently not carriers (based on three negative stool cultures).
4) 59 healthy individuals, age 16–46 years.

Of the 36 chronic carriers, 27 (75%) had Vi reciprocal titers of ≥160 (see Table 6), whereas only 8% of the 388 noncarrier women ($p < 0.001$) and 3% of 59 health subjects (who had no bacteriological screening) ($p < 0.001$) had titers ≥160. The frequency of titers ≥160 in patients with acute typhoid fever (38%) was also significantly lower than that in chronic carriers ($p < 0.005$). The geometric mean titer in the chronic carriers was significantly ($p < 0.001$) higher than that in any of the other groups (Table 6). Using the 388 culture-negative women as negative controls, a Vi antibody titer of ≥160 was 75% sensitive and at least 92% specific in detecting chronic carriers.

In Santiago, the predictive value (*35*) of a

Table 6. Prevalence of Vi antibody[a] in chronic *Salmonella typhi* carriers, acute typhoid fever and healthy populations in Santiago, Chile.

Group	Characteristics	N	Reciprocal geometric mean titer	% with reciprocal titer		
				≤40	80	≥160
Chronic *S. typhi* carriers	92% females 17–59 years	36	296	14	11	75
Acute typhoid fever patients	Both sexes 18–30 years	29	53	48	14	38
Noncarriers with typhoid fever 1–4 years earlier	100% females 24–62 years	388	21	85	7	8
Healthy Chileans	Both sexes 16–46 years	59	16	85	12	3

[a] Measured by passive hemagglutination using highly purified Vi antigen.

titer ≥ 160 is at least 8% in the general adult population, 16% in women 40 years and older, and 3% in women 25 years and older with history of confirmed typhoid fever. The practical application of the simple passive hemagglutination assay with highly purified Vi antigen to detect chronic *S. typhi* carriers in an endemic area like Santiago, Chile depends greatly on its predictive value. Since the predictive value is high in populations with high chronic *S. typhi* carrier rates (such as older women), screening high-risk groups of the population is warranted as part of a program to control typhoid fever. For this reason, systematic serological screening of foodhandlers in Santiago schools (90% of whom are women over 30 years of age) has been initiated.

Treatment of Chronic Typhoid Carriers

When a chronic *S. typhi* carrier is identified, interventions must be initiated to minimize the chance for transmission of *S. typhi* by the carrier to susceptibles. Health education, including counseling on personal hygiene and food preparation techniques, is fundamental. Ideally, however, therapy to eradicate the chronic carrier state is desired. The currently recognized "gold standard" of therapy involves cholecystectomy followed by several weeks of antibiotic (usually ampicillin or amoxicillin) therapy. Obviously, such a therapeutic regimen involving major abdominal surgery is unsuitable as a routine public health intervention in endemic areas where the prevalence of carriers is high. Thus, for decades, an alternative, nonsurgical therapeutic regimen has been sought to successfully cure chronic *S. typhi* carriers.

Italian investigators (*36*) reported that two weeks of intravenous ampicillin (1.0 g q 8 h) successfully cured 19 chronic *S. typhi* biliary carriers. However, intravenous antibiotic therapy precludes self-administered domiciliary treatment and thus is also not practical for public health use. The advent of amoxicillin made available a superbly absorbed analog of ampicillin that provides serum levels following oral administration that were previously achievable only with parenteral administration of ampicillin. Furthermore, like ampicillin, amoxicillin is concentrated in bile. Nolan *et al.* (*37*) recognized that these features of amoxicillin made it worthy of evaluation as a nonsurgical treatment for the chronic *S. typhi* carrier state. Nolan *et al.* (*37*) treated 15 chronic *S. typhi* biliary carriers with oral amoxicillin (2.0 g three times daily) for 28 days. Long-term cures were observed in 9 of 10 carriers who were able to complete the month of therapy.

Encouraged by these preliminary results of Nolan *et al.* (*37*), we proceeded to evaluate a 28-day course of oral amoxicillin (2.0 g three times daily) plus probenecid (0.5 g three times daily) in treatment of chronic *S. typhi* carriers in Santiago, Chile (C. Lanata *et al.*, unpublished data). Twenty-eight confirmed chronic carriers (27 females) began the course of therapy. Antibiotic and probenecid for each day of therapy were provided in small vials. Medication was taken at home or at work and the times of dosing were recorded by the patient in a small diary. Patients were visited in their homes at least once weekly on a scheduled basis. In addition, random unscheduled visits were made at least once weekly. At both scheduled and surprise visits, urine specimens were collected for measurement of amoxicillin levels.

Two of the 28 patients were unable to complete the course of amoxicillin therapy because of severe allergic reactions which were manifested in the first or second day of therapy. Of the remaining 26 carriers who successfully completed the 28-day course of amoxicillin and probenecid, many complained at one time or another of mild diarrhea, rash, nausea, abdominal discomfort, or gastritis. In no instances were the symptoms sufficiently severe to cause discontinuation of therapy.

The success of therapy was monitored by means of stool cultures and bile cultures (obtained by string capsule device) at monthly in-

tervals following completion of therapy. This nonsurgical, ambulatory, domiciliary oral treatment regimen resulted in long-term (1 year) cure of 15 of the 26 carriers (58%). When failure occurred it was usually evident within the first 6 weeks following cessation of therapy. Thirteen of the 26 carriers have had radiological evaluation of their gall bladder function; cholelithiasis, failure of the gall bladder to fill during cholecystogram, or other pathology was present in 13 of 13 carriers examined so far.

A cure rate of 58% with a domiciliary oral antibiotic regimen, despite the presence of gall bladder dysfunction, is encouraging news for treating an individual patient, since there is a greater than ever chance of cure without surgery. However, such a cure rate is too low to advocate its use in public health programs. Therefore, we are continuing to seek an antibiotic regimen that will cure at least 80% of carriers, even with gallstones, without cholecystectomy.

Large-Scale Field Trials of the Ty21a Live Oral Typhoid Vaccine

The live oral typhoid vaccine, Ty21a, developed by Germanier and coworkers (*38*) represents a potentially major breakthrough for the control of typhoid fever by immunization. In the initial clinical studies with this live attenuated *Salmonella typhi* oral vaccine in North American volunteers, it was shown to cause no adverse reactions and to be genetically stable and highly protective (*39*).

The first field trial with Ty21a was carried out in Alexandria, Egypt where approximately 16,000 6 and 7 year old schoolchildren were given three doses (10^9 viable vaccine organisms per dose) within one week (*40*). Individual doses of lyophilized vaccine contained within small glass vials were reconstituted on the spot, and the children were vaccinated a few minutes after they chewed a tablet containing 1.0 g of $NaHCO_3$ to neutralize gastric acid. An equal number of children ingested placebo. In this trial, the vaccine provided 96% efficacy for at least three years in an area where the incidence of confirmed typhoid fever in the control group was 40 per 10^5 schoolchildren.

Stimulated by highly encouraging results of the Egyptian trial, a collaborative effort was undertaken to carry out field trials of Ty21a in Santiago, Chile to obtain new information and to evaluate the possible use of this vaccine as a public health intervention to control endemic typhoid fever in Chile.

Two separate field trials of efficacy of one Ty21a vaccine have been undertaken in Santiago, Chile in Area Norte (Trial 1) and Area Occidente (Trial 2). Results of these controlled field trials are summarized below.

Area Norte Trial

The goals of the first Chilean field trial in the Northern Administrative Area (Area Norte) included:

1) To evaluate the efficacy of a new formulation of Ty21a vaccine (enteric-coated capsules) that is more amenable to mass vaccination, since $NaHCO_3$ pretreatment is unnecessary.

2) To investigate the efficacy of fewer (one or two) doses of vaccine than were used in the Alexandria, Egypt field trial.

3) To assess the efficacy in an area of particularly high endemicity and force of infection.

Parents of 91,954 of the 137,697 schoolchildren in Area Norte gave permission for their children to participate in the trial. These children were randomized so that in May and June, 1982, 31,762 received two doses of placebo, 32,707 received one dose of vaccine and one of placebo, and 27,485 received two doses of vaccine (one week apart). The remaining 45,743 unvaccinated children were considered as a separate "contact" control group.

A summary of 24 months of surveillance is contained in Table 7. Briefly, two doses of the vaccine stimulated a moderate degree (59%) of protection which continued over two typhoid seasons. In Table 8 the results are di-

Table 7. Area Norte field trial. Efficacy of one and two doses of Ty21a live oral typhoid vaccine given in enteric-coated capsules.

Summary of 24 months of surveillance. 1 July 1982 to 30 June 1984				
Vaccine group	No. of children	No. of cases[a]	Incidence (per 10^5)	Percent vaccine efficacy
2 doses	27,485	40	145.5[b]	59
1 dose	32,707	85	259.9[c]	26
Placebo	31,762	112	352.6	
Not vaccinated	45,743	147	3211.4	

[a] Bacteriologically confirmed by blood or bone marrow culture.
[b] vs [c], $p < 0.2$.
[b] vs [c], $p < 0.04$.
[c] vs [b], $p < 0.003$.

Table 8. 24 Months of surveillance of the Area Norte field trial. Efficacy of two doses of enteric-coated formulation of Ty21a oral typhoid vaccine.

	Placebo (31,762)		Two doses (27,485)		
Surveillance period	No. of cases[a]	Rate $\times 10^5$	No. of cases	Rate $\times 10^5$	Percent vaccine efficacy
1982 July-Sept.	1	3.1	0	0	100
Oct.-Dec.	15	47.2	4	14.6	69
1983 Jan.-Mar.	37	116.5	10	36.4	69
Apr.-June	16	50.4	16	58.2	0
July-Sept.	4	12.6	1	3.6	71
Oct.-Dec.	14	44.1	1	3.6	92
1984 Jan.-Mar.	19	59.8	6	21.8	64
Apr.-June	8	25.2	3	10.9	57

[a] Bacteriologically confirmed by blood or bone marrow culture.

vided into three-month periods of observation. In this analysis one notes that there was one three-month period (April-June, 1983) during the 24 months of surveillance when the protective effect of the vaccine appeared to have been overwhelmed (Table 8); in all other periods vaccine efficacy exceeded 57% (Table 8).

Other important observations from the Area Norte field trial include:

1) The vaccine caused no significant adverse reactions in 60,000 vaccinated children.

2) The enteric-coated formulation was found to be highly practical and well suited to mass vaccination.

3) The annual incidence of culture-confirmed typhoid fever in the placebo control group in the first year of surveillance in Area Norte was 214 $\times 10^5$, a rate more than five times higher than the rate in the control group in the Egyptian trial!

4) One dose of vaccine gave much less protection (30%) than two doses of vaccine (59%).

5) As shown in Table 9, two doses of vaccine also provided moderate protection against *S. paratyphi* B infection. This makes sense since protection with Ty21a and other live *Salmonella* vaccines is known to be related to the lipopolysaccharide O antigen (*41, 42*). The O antigens of *S. typhi* and *S. paratyphi* B are related.

Table 9. Efficacy of Ty21a attenuated *Salmonella typhi* vaccine against *S. paratyphi* B disease.

Vaccine group	No. of children	No. cases of paratyphoid B*	Incidence per 10^5	Percent vaccine efficacy
2 doses	27,485	6	21.8[a]	51
1 dose	32,707	10	30.6[b]	31
Placebo	31,762	14	44.1[c]	
Not vaccinated	45,743	23	50.3	

* Bacteriologically confirmed by blood or bone marrow culture.
[a] vs [c], $p = 0.2$.

Area Occidente Field Trial

Based on results of the field trial in Area Norte which showed only moderate efficacy with two doses of enteric-coated vaccine and an apparent overwhelming protection in vaccinees during one three-month period, a second field trial was initiated in Area Occidente of Santiago. In this trial 141,127 children of consenting parents (representing 95% of all schoolchildren in Area Occidente) were randomized to one of five groups to receive:

Group 1—Three doses of vaccine in enteric-coated capsules given within one week.

Group 2—Three doses of vaccine with $NaHCO_3$ given within one week. The commercial gelatin capsule/$NaHCO_3$ formulation was used, which consists of two gelatin capsules each containing 0.5 g of $NaHCO_3$ and one gelatin capsule containing lyophilized vaccine.

Group 3—Three doses of enteric-coated vaccine with an interval of three weeks between the doses.

Group 4—Three doses of the gelatin capsule/$NaHCO_3$ formulation with an interval of three weeks between the doses.

Group 5—Three doses of placebo.

The design of this trial was intended to allow a direct comparison of two different formulations of vaccine as well as two different immunization schedules. We would like to have included the Egyptian formulation as one cell in this trial; however, that formulation was not available (Table 10). The Egyptian formulation was prepared only for the Alexandria trial and then was replaced by the gelatin capsule/$NaHCO_3$ formulation, which became available commercially in many countries of the world.

Table 10. Field trial formulation of Ty21a live oral typhoid vaccine.

Vaccine formulation	$NaHCO_3$	Where used
Lyophilized vaccine in glass vials under vacuum	1.0 g tablets	Alexandria, Egypt
Lyophilized vaccine in enteric-coated capsules	None	Santiago, Chile (Area Norte and Occidente)
Lyophilized vaccine in gelatin capsules	2 gelatin capsules each with 0.5 g	Area Occidente, Santiago, Chile

Similarly, the more practical enteric-coated formulation was made in two special lots for the Area Norte and Area Occidente trials in Chile (Table 10): this enteric-coated formulation was not commercially available at that time.

Results of the Area Occidente field trial are shown in Table 11. The clear-cut results allow the following conclusions to be drawn:

1) The extraordinary safety of Ty21a oral vaccine was once again shown in 107,450 Area Occidente schoolchildren who ingested at least two doses of vaccine between July and September, 1983.

2) The enteric-coated formulation was found to be significantly more protective than the gelatin capsule/$NaHCO_3$ formulation.

3) The long-interval immunization schedule (21 days between doses) gave no greater protection

Table 11. Efficacy of Ty21a oral typhoid vaccine in Area Occidente after 10 months of surveillance: comparison of two different formulations and immunization schedules.

Three doses within one week	No. of children	No. of cases*	Rate 10^4	Percent vaccine efficacy
Enteric-coated	22,170	7	31.6[a]	74
gelatin/$NaHCO_3$	22,379	24	107.2[b]	12
Three doses, 21 days between each dose				
Enteric-coated	21,598	8	37.0[a]	70
gelatin/$NaHCO_3$	21,541	19	74.2[c]	39
Placebo	27,793	34	122.3[b]	
Not vaccinated	14,962	16	106.9	

*Bacteriologically confirmed by blood or bone marrow culture.
[a] or [c] vs [b], p 0.002.
[b] vs [b], p 0.005.

than when all doses of vaccine were given within one week.

4) The level of protection achieved with three doses of enteric-coated vaccine was approximately 70–75%.

5) Some children were absent during one day of vaccination and consequently received only two doses of vaccine. The numbers of such children are small and these children may represent a skewed group at different risk from those who were not absent on any vaccination days. Nevertheless, it seemed worthwhile to compute the incidence rate in the children who received only two doses and compare it to those who received three doses of vaccine or placebo. In Table 12, the incidence of typhoid fever in all children who received three doses of enteric-coated vaccine (both short and long immunization schedules) is compared with the rate in all recipients of two doses of enteric-coated vaccine and with the incidence rate in placebo children. In this analysis, no evidence is found for lesser immunity in recipients of two doses of vaccine. Again one must stress that the groups of two- and three-dose recipients were not randomized and therefore comparison may be inappropriate.

A Third Santiago Field Trial

Based on results of the Area Occidente field trial, it is obvious that the enteric-coated formulation is the formulation of choice and that a short interval immunization schedule is satisfactory. However, it is still unclear whether there is a difference in the protection conferred by two versus three doses given within eight days. Nor is it clear whether a fourth

Table 12. Comparison of efficacy of three versus two doses of enteric-coated Ty21a vaccine in the Area Occidente field trial (10 months of surveillance).

Vaccine	No. of children	No. of cases	Incidence per 10^4	Percent vaccine efficacy
Enteric-coated* 3 doses	43,768	15	34.3	72
Enteric-coated* 2 doses	9,920	2	20.2	83
Placebo	27,793	34	122.3	

* Includes both short- and long-interval schedules.

dose might significantly enhance protection. The answers to these questions were to be sought in a third field trial that began with vaccinations in October 1984. In this trial 285,000 schoolchildren in Area Sur and Area Central of Santiago were randomized to receive two, three, or four doses of enteric-coated vaccine given within eight days. For ethical reasons, no placebo group was included; thus only relative efficacy will be determined.

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Implementation Phase

During the past five years, the epidemiology of endemic typhoid fever has been intensively studied and various interventions evaluated. We are now at the point of definitive

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APPENDIX B

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Case-control study to identify risk factors for paediatric endemic typhoid fever in Santiago, Chile

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Typhoid fever is an important endemic health problem in Santiago, Chile. Its incidence has more than doubled in recent years, during which access to potable water and sewage disposal in the home became almost universal in the city. A matched case-control study was carried out to identify risk factors and vehicles of transmission of paediatric typhoid fever; 81 children in the 3–14-years age group with typhoid fever were compared with controls, matched with respect to age, sex, and neighbourhood. It was found that case children more frequently bought lunch at school and shared food with classmates. Also, case children more often consumed flavoured ices bought outside the home; none of 41 other food items considered in the study was associated with a higher risk of typhoid fever. Only two food handlers for cases and one for controls were positive for Salmonella typhi, *indicating that persons preparing food solely for their own family were not the main source of* S. typhi *infection. Rather, the risk factors identified in this study are consistent with the hypothesis that paediatric endemic typhoid fever in Santiago is largely spread by consumption of foodstuffs that are prepared outside the individual's home and are shared with or sold to children.*

Typhoid fever is an endemic health problem in Chile, presenting some interesting and mostly unexplained epidemiological features. The illness has a marked seasonality with a peak during the summer months and the highest incidence is in children in the 8–13-years age group (*1*, *2*). Furthermore, its incidence is high in children from both low and high socioeconomic groups, even those who live under apparently nearly optimum sanitary conditions (*3*).

Significant improvement has been achieved in reducing the mortality rate of typhoid fever in Chile from 12 per 10 000 inhabitants in the 1940s to less than 1 per 10 000 in the late 1970s; however, over the same period the morbidity rate has increased from 50 per 10 000 inhabitants to 100 per 10 000 (*4*). Paradoxically, this increase in morbidity occurred during a period in which access to potable water and sewage disposal in the home increased and became almost universal in urban areas (*3*, *5*). Furthermore, during this time there was a striking reduction in the frequency of most other communicable diseases in Chile (*6*).

Little is known about the routes of transmission for typhoid fever in Santiago. The two principal hypotheses proposed suggest contamination of food (*a*) by foodhandlers who are asymptomatic carriers of *Salmonella typhi* (*2*) or (*b*) by the irrigation of fruit and vegetables with sewage-contaminated water (*7*, *8*). As far as the first hypothesis is concerned it should be noted that the prevalence of cholelithiasis in Chile is one of the highest in the world (*9*) and that this, together with the endemic presence of typhoid fever in the country (*1–4*), produces a high rate of chronic biliary carriage. It has been estimated that there are nearly 30 000 such carriers of *S. typhi* in Santiago (a prevalence rate of 694 per 10 000 (*10*)). With regard to the second hypothesis, sewage in Santiago is discharged untreated into the Mapocho river and a large canal; water drawn from these sources is used to irrigate crops, such as lettuce and celery, which are grown near the city (*7*). High faecal

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coliform counts have been measured in this water, and *S. typhi* organisms have been isolated from it (*8*).

The aim of the present study was to identify risk factors and vehicles of transmission of typhoid fever in the eastern part of Santiago, an area that accommodates families of all economic strata, but mostly middle- and high-income persons living in modern housing.

MATERIALS AND METHODS

A matched case–control study was conducted from December 1980 to June 1981. Cases selected were children of either sex in the age group 3–14 years who lived in the eastern area of Santiago and who were diagnosed as having typhoid fever as confirmed by blood and/or bone marrow cultures that were positive for *S. typhi*. Children were diagnosed and treated at the Calvo McKenna Hospital (serving mainly low and middle socioeconomic groups) or by 20 paediatricians who care more for middle and upper socioeconomic groups in their private practices. A surveillance system was established to ensure that two blood cultures were obtained from each child with suspected typhoid fever at either the hospital or the private practices. Blood was cultured in a medium of supplemented peptone broth,* and processed by standard methods (*11*, *12*).

Controls were children of the same sex and age (± one year of age) as the cases, and lived in the same neighbourhood. They were identified by following a standardized route which started at the home of the case. Controls who had had a febrile illness suggestive of typhoid fever during the four weeks prior to their participation in the study were excluded and a new control was selected. Once an appropriate control was identified, return visits to the neighbourhood were made until complete information was obtained.

Two public health nurses filled in questionnaires concerning cases and their matched controls, and the answers given by the children were verified by interviewing their mothers. For young children, the questions were answered by their mothers. Cases and matched controls were interviewed by the same nurse. The questionnaire explored the following areas: socioeconomic level (type of house construction and ownership; number of rooms, beds, and persons in house; and ownership of car); sanitary conditions at home (existence of water source and bathroom facilities); food and drink consumption (42 items), both at home and outside (for the two weeks prior to the onset of illness in cases and over the same period for the matched controls); existence of cooks and/or maids at home and their role in food preparation; frequency of eating food from street vendors, in restaurants, or at school; history of gall bladder disease among the food handlers at home; contact with known cases of typhoid fever (in the two months preceding the study); and travel and swimming activities (in the month prior to onset of illness for cases and in the same period for the matched control).

For both cases and controls two stool samples were obtained from the primary food handler in the household. The initial sample was obtained by rectal swab at the time of the interview and placed in Cary-Blair transport medium and cultured the same day. The second sample was a stool obtained during the morning of the following day which was kept refrigerated and was cultured within six hours. Faecal samples were cultured on *Salmonella-Shigella*, MacConkey's, or bismuth sulfite agars, either directly or after selenite enrichment for 24 hours; colonies harbouring *Salmonella* or *Shigella* bacteria were identified using standard techniques (*12*).

Statistical analysis of the results for matched pairs was carried out using the McNemar test (*13*) if the outcome was dichotomous, and a similar test derived by Fleiss (*13*) if multinomous.

RESULTS

Eighty-one cases met the criteria for inclusion in the study; 67 were from Calvo McKenna Hospital and 14 were from the practices of private paediatricians in Santiago. The largest number of cases were 6-year olds, with relatively few below this age and fairly uniform frequencies above it; there were equal numbers of male and female children (Fig. 1).

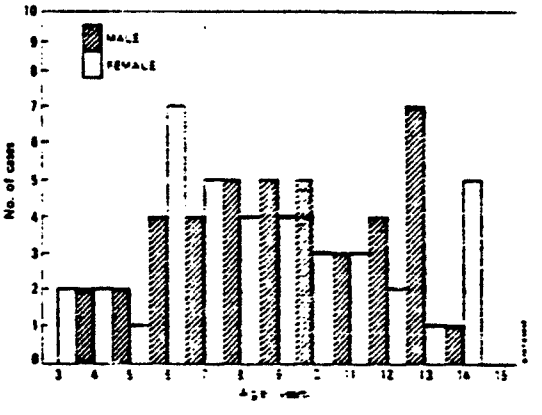

Fig. 1. Age and sex distribution of 81 paediatric cases of typhoid fever in the eastern area of Santiago, Chile, over the period December 1980–June 1981

* From Becton Dickinson and Co., Oxnard, CA 93030, USA.

Table 1. Association of selected risk factors with paediatric typhoid fever in Santiago, Chile

	Case:Control pairs					
	Yes:Yes	Yes:No	No:Yes	No:No	Significance	Relative risk
History of typhoid fever in a relative	0	16	5	58	$\chi^2=4.8$, $P<0.05$	3.2
Bought lunch at school*	2	12	3	35	$\chi^2=4.3$, $P<0.05$	4.0
Travel outside Santiago	12	8	21	40	$\chi^2=5.0$, $P<0.05$	0.4
Swimming in a lake	9	0	8	72	$\chi^2=6.1$, $P<0.025$	C
Swimming in a pool	10	7	18	46	$\chi^2=4.0$, $P<0.05$	0.4

* Analyses confined to cases that occurred while school was in session.

Rectal swabs were obtained from 78, and stool samples from 77, of the 81 domestic food handlers for cases; 2 food handlers were positive for *S. typhi*, including the mother of one case and the cook (female) of another. Swabs were also obtained from 81, and stools from 71 food handlers of controls. One of these food handlers was positive for *S. typhi*; this was the mother of a control child whose own children were negative for *S. typhi* but who prepared flavoured ices that she sold to neighbourhood children, including the case matched with her child. The ages of these food handlers were 34, 37 and 55 years; none of them had a history of typhoid fever, but two had a history of biliary colic and one was known to have gallstones. Seven additional food handlers had stool cultures positive for other enteropathogens, including *S. paratyphi* B (3), other salmonellae (3) and *Shigella* (1).

The families of cases and matched controls had the same number of persons in the home, and all had household electricity, sewage disposal facilities, sinks in bathroom and kitchen, and a source of water, or owned a refrigerator or an automobile. The relatives of cases (usually cousins), but not friends, were more frequently (relative risk 3.2) reported ill with typhoid fever during the two months preceding the study (Table 1). Cases more frequently (relative risk 4.0) ate lunch bought at school than controls (Table 1), but both groups ate food from street vendors, school kiosks, and restaurants. In addition, cases more frequently shared food at school with friends (Table 2). This increased risk was particularly apparent for children who shared food in this way three or more times per week (relative risk 6.0) (Table 2), and seemed to be more important for children who brought food to school (relative risk 10/2, $\chi^2=5.4$, $P<0.05$) than for children who purchased school food (relative risk 2/0, not significant).

Controls travelled away from Santiago more frequently than did cases in the month before the onset of illness in the matched case (Table 1). This lower risk for controls who travelled seemed to hold during both summer (relative risk 5/10) and non-summer (relative risk 3/10) months. Controls also swam more frequently in a lake or pool than cases (Table 1).

The consumption patterns of cases for 42 fruits and vegetables and other food products were obtained for two weeks prior to onset of their illness and for the same period in matched controls. Consumption of

Table 2. Frequency of sharing food with friends among typhoid fever cases and matched controls*

	No. of matched controls sharing food		
No. of cases sharing food	≥3 times/week	1-2 times/week	Never
≥3 times/week (12)†	0	9	3
1-2 times/week (29)	2	15	12
Never (34)	0	11	23

* $\chi^2=7.3$, $P<0.05$ (2 degrees of freedom).

† Figures in parentheses are totals.

Table 3. Frequency of consuming purchased flavoured ices by typhoid fever cases and matched controls*

Case consumption	Control consumption		
	≥3 times/week	1–2 times/week	Never
≥3 times/week (34)†	15	8	13
1–2 times/week (11)	4	5	2
Never (34)	3	5	26

* $\chi^2 = 7.3$, $P < 0.05$ (2 degrees of freedom).

† Figures in parentheses are totals.

purchased flavoured ices was associated with a higher risk of typhoid fever, particularly among children consuming such ices three or more times per week (relative risk 3.0) (Table 3). Consumption of flavoured ices made in the home was not associated with typhoid fever (relative risk 5/9). No association was found between consumption of the other food items, including suspected vehicles or groups of these items, and development of typhoid fever. For example, the relative risk associated with consumption of lettuce was 14/19 (0.74) and strawberries 23/19 (1.2). One food item, *mote con huesillos* (a local drink made from corn and apricots), was associated with a significantly lower risk of typhoid fever (relative risk 4/16, $\chi^2 = 9.9$, $P < 0.01$).

DISCUSSION

The epidemiology of endemic diseases is frequently complex. Unlike common-source outbreaks, many vehicles, each responsible for a few cases, may be involved, and this might be the situation regarding typhoid fever in Santiago, Chile. The present study incriminated only one food item but identified a number of factors associated with a higher or lower risk of developing typhoid fever.

Since more than 70 variables were investigated, it can be expected that a few of them are statistically significant by chance alone at a P level of less than 0.05. In some instances, however, the statistical significance of the association was greater than 0.05. Furthermore, some risk factors were corroborated by statistical significance of two or more related variables, e.g., travel outside Santiago and swimming in lakes or pools, usually outside the city. Since these are related variables, the finding of all three to be important factors suggests that this was not due to chance. Cases were probably matched so closely with controls from the same neighbourhood for socioeconomic status that potential risk factors associated with wealth, education, or place of residence may have been overlooked. On the other hand, this study design allowed examination of important risk factors without the potentially confounding effects that may have arisen had cases and controls differed in socioeconomic status.

The study identified flavoured ices as a vehicle of transmission of typhoid fever among children in Santiago; however, the precise means of contamination of the ices has not yet been established. One possibility is that the water used to prepare them was contaminated, but the almost universal household access to water of good quality in study families and in Santiago in general (*5*) and the failure of this study to implicate the water source as an important risk factor suggest that this is unlikely. Another possible explanation is that *S. typhi* carriers contaminated the containers, the water, or the ice in their homes while preparing the flavoured ices for sale. In this respect, it is pertinent that we identified an *S. typhi* carrier who had prepared and sold flavoured ices to a child who subsequently developed typhoid fever. The lack of significance, as a risk factor, of flavoured ices made and consumed at home further emphasizes that it is the preparation of the food item by carriers outside the home that is important.

Consuming lunch bought at school cafeterias and sharing food with classmates who did not buy lunch at school were both associated with a higher risk of typhoid fever. Although we did not detect any clustering of cases in particular schools, these data suggest that one transmission route of *S. typhi* may be consumption at school of food that was prepared on the school premises, brought by their children from home, or bought from food vendors. It is also possible that certain children have a tendency to eat food prepared outside their own homes, thus exposing themselves to food prepared by a wide variety of persons, some of whom could be chronic carriers of *S. typhi*. The higher frequency of reported (but undocumented) typhoid fever among relatives of cases compared to those of controls could be due to reporting bias; however, it may indicate that the case and the relative had shared a common food exposure.

The reduced risk of typhoid fever among children who travelled out of Santiago may be a marker of socioeconomic status but could also indicate that they were removed from the source of infection, being safer away from the city. The incidence rates of typhoid fever in the popular holiday resorts are much lower than those in Santiago (*2*).

This study indicates that food handlers who prepared food solely for their own families were not

the main source of *S. typhi* infection. Cultures of two stool samples should identify most asymptomatic carriers, and only two carriers were found in the homes of the 81 cases. Furthermore, this finding was corroborated by a later study of family members of typhoid fever patients in Santiago (*15*). Using three stool cultures and measurement of Vi antibodies (*16*), only one chronic carrier was found among the family members of 24 patients with typhoid fever. Although chronic carriers are undoubtedly important in the transmission of *S. typhi*, all these studies indicate that such carriers within the household could account for only a small fraction of typhoid fever cases. The risk factors identified in the present study are consistent with the hypothesis that endemic typhoid fever in Santiago is largely spread by exposure to food items that are prepared in schools, private homes, or by food vendors and that are shared with or sold to children. From this study, it cannot be determined whether contamination of these items with *S. typhi* was a result of their preparation by chronic *S. typhi* carriers or because the raw foodstuffs were contaminated by *S. typhi* from Santiago sewage. Further epidemiological and bacteriological studies are planned to resolve this issue.

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RÉSUMÉ

ETUDE CAS-TÉMOINS EN VUE DE DÉTERMINER LES FACTEURS DE RISQUE DE LA FIÈVRE TYPHOÏDE ENDÉMIQUE DE L'ENFANT À SANTIAGO DU CHILI.

La fièvre typhoïde est un problème de santé endémique au Chili, qui atteint un paroxysme pendant l'été et sévit surtout chez les enfants de 8 à 13 ans. A Santiago, l'incidence de la fièvre typhoïde a doublé ces dernières années, bien que la quasi-totalité des ménages aient accès à l'eau potable et à l'évacuation des eaux usées; l'incidence est élevée, tant dans les quartiers pauvres que dans les quartiers riches de la ville. Une étude de cas et de témoins appariés a été entreprise en vue de déterminer les facteurs de risque et les véhicules de transmission de la maladie. Quatre-vingt un enfants de trois à quatorze ans, souffrant de fièvre typhoïde confirmée par analyse bactériologique ont été comparés à des sujets témoins appariés par âge, sexe et voisinage. L'étude a révélé des facteurs de risque qui correspondent à l'hypothèse selon laquelle, à Santiago, la consommation de produits alimentaires partagés par les enfants ou vendus à ceux-ci hors de la maison est dans une large mesure responsable de la propagation de la maladie. La contamination de ces produits par *Salmonella typhi* peut être due à leur préparation par des porteurs chroniques de *S. typhi* ou au fait que les denrées entrant dans leur composition contiennent *S. typhi* par suite d'une pollution par les eaux usées.

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APPENDIX C

Am. J. Trop. Med. Hyg., 33(6), 1984, pp. 1195–1202

TYPHOID FEVER IN SANTIAGO, CHILE: A STUDY OF HOUSEHOLD CONTACTS OF PEDIATRIC PATIENTS*

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Abstract. We obtained clinical, epidemiological, and laboratory data (including three stool cultures) from 155 (96%) of 161 household contacts of 24 patients <16 years old with culture-confirmed typhoid fever; these 24 patients represented approximately 40% of such patients seen in three hospitals in Santiago during a 12-week period. A chronic typhoid carrier was identified in only one household, with concurrent or secondary cases seen in two other households. When index cases were matched with household members nearest in age, no specific risk factors for illness could be identified. There was evidence of generalized exposure to enteric pathogens within these households, with nine persons from seven different households culture-positive for non-typhoidal *Salmonella*, and nine, from eight different households, culture-positive for *Shigella*; transmission of these pathogens within households did not appear to be common since no household had more than one family member with the same serotype or species of either pathogen.

Typhoid fever is endemic in Santiago, Chile, where an average of 170 cases per 100,000 population per year was reported for the years 1977–1981.[1,2] The incidence of typhoid fever is highest among children 3–13 years of age and relatively low among children ≤4 and adults aged ≥25 years. Over 75% of cases occur during the summer and early fall (December–May). Despite generally high standards of sanitation and medical care, the incidence rate for typhoid in Santiago, rather than declining, has almost doubled during the past decade. The reasons for the large number of cases in the city and for the recent increase in incidence are still not well understood;[3,4] contributing factors may include a high chronic carrier rate (estimated at 694 carriers per 10^5 population[3]), problems with food sanitation exacerbated by recent deregulation of the local food service industry,[5] and use of untreated waste water for irrigation of crops in the summer.[4]

We sought to examine these and other factors by studying households in Santiago in which a child had recently been diagnosed as having typhoid fever. Within households we attempted to identify chronic typhoid carriers and possible concurrent cases; we also attempted to identify risk factors for infection, including patterns of food consumption away from the household and preferences for specific "high risk" foods such as raw vegetables. Although our study was designed to evaluate infections with *Salmonella typhi*, the culture techniques used allowed us to identify other *Salmonella* and *Shigella* species in stool samples; as we found a relatively high rate of stool carriage of both non-typhoidal *Salmonella* and *Shigella*, clinical and epidemiological data related to these pathogens have been included in our analysis.

METHODS

Laboratory records in three Santiago hospitals (Hospital Felix Bulnes, Hospital San Juan de Dios, and the Infectious Diseases Hospital) were reviewed daily or every other day during a 12-week period in March, April, and May 1983. Efforts were made to visit the households of all patients <16 years of age who had culture-confirmed typhoid fever; if more than one case was identified on a single day, the household of the youngest patient identified was visited. A standardized questionnaire was administered to each index case and to each household member; data

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requested included the occurrence of symptoms such as fever or diarrhea, frequency of eating food away from the household (at school, at the homes of relatives or friends, or purchased from street vendors), recent travel history, history of exposure to persons with typhoid outside of the family, and a limited list of food preferences (ice cubes purchased outside of the home; raw vegetables, including lettuce, cabbage, celery, and tomatoes; fresh fruit; and fresh seafood). A second questionnaire related to the physical characteristics of the household was administered to the head of each household.

Three stool cultures were collected from each household member, with efforts made to obtain cultures on days 1, 2, and 7 after the initial household visit. Persons were instructed to sample a fresh stool with a sterile swab and place the swab in a vial containing Cary-Blair transport medium. At the Enterics Laboratory of the Institute of Public Health swabs were directly plated on SS, MacConkey's and bismuth sulfate agar, and then placed in selenite enrichment for 24 hours. Colonies suspicious for *Salmonella* or *Shigella* were identified using standard techniques.[6,7] All isolates were serotyped or speciated, or both, by the Reference Laboratory of the Institute of Public Health. Plasmids were identified in all *Shigella* isolates using an alkaline plasmid extraction technique.[8]

At the time of the first household visit a blood sample was collected from each household member over 10 years of age. Serum was frozen at −20°C until the conclusion of the study, at which time samples were assayed for Vi antibodies by passive hemagglutination,[9] using purified *S. typhi* Vi antigen (kindly provided by John Robbins, Bureau of Biologics, Bethesda, MD); titers ≥1:160 were regarded as positive.[10] Four water samples for fecal coliform and chlorine analysis were collected on successive visits from the kitchen water supply in each household.

RESULTS

Families of 24 patients (representing approximately 40% of eligible families) were contacted and agreed to participate in the study. The average age of these patients was 10 years (standard deviation ± 4 years), compared to an average age of 8.9 years for all reported typhoid cases in Santiago among patients <16 years old. Excluding the index cases, a total of 161 persons lived

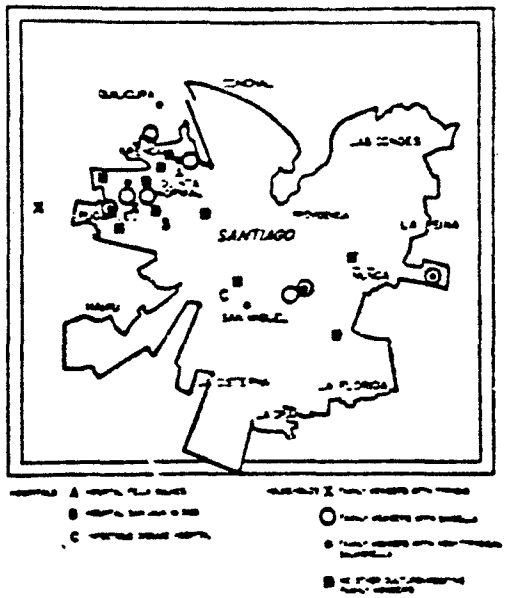

FIGURE 1. Santiago metropolitan area: Location of hospitals and households studied.

in the 24 households: complete clinical, epidemiologic, and laboratory data were obtained for 155 (96%). Thirty persons, in 18 households, were <10 years of age; 14, in 10 households, were ≤4 years of age.

Households included in the study were concentrated in the western part of the city near the three hospitals surveyed (Fig. 1). Households had an average of 2.4 persons per room (range 0.9–4.6). Nineteen (79%) of the households were connected to the city sewerage system, all had electricity, and all but one had municipal water. Because of delays in obtaining initial cultures and in confirming the identification of isolates, the average time between a patient's onset of symptoms of our initial visit to the household was 21.5 days (range 11–37 days).

Salmonella typhi

We identified a chronic *S. typhi* carrier on only one household. The carrier, the 38-year-old stepfather of the index case, gave a history of having had typhoid fever in 1960. He was asymptomatic at the time of the study, with multiple positive stool cultures for *S. typhi*; he had a strongly positive Vi antibody titer (1:640).

Apparent concurrent or secondary cases were identified in two households. In the first, all six household members were culture positive for *S. typhi*; four were symptomatic, and two were hospitalized. No household member had a history of typhoid, and none had an elevated Vi antibody titer. The water supply for the household came from a well which was not chlorinated, and water samples had consistently high fecal coliform counts. In a second household, the index case's 28-year-old sister developed fever and gastrointestinal complaints, with positive stool cultures for *S. typhi*, 5 weeks after onset of symptoms in the index case; she had no previous history of typhoid and did not have an elevated Vi antibody titer.

Thirty-six (60%) of 60 culture-negative household members <16 years of age ate food outside of the household at least once during an average week, compared to 19 (86%) of 22 index cases (excluding 2 index cases for whom no matched household controls were available) ($P = 0.01$, by the method of Pike and Morrow for matched cases and variable numbers of controls.[11]) However, index cases were significantly older than household controls (mean age 10 years vs. 6.5 years) ($P = 0.03$, Mann-Whitney U test), and it was possible to show within the control group that older children were more likely than younger children to eat away from home ($P = 0.01$, Mann-Whitney U test). When index cases were matched with the culture-negative household member closest in age (but <16 years old) the difference in food consumption outside of the household was not significant ($P > 0.05$, McNemar test for matched cases and controls). No other risk factors for illness could be identified, using both age-matched and non- aged-matched controls.

Serum samples were obtained from 122 household members. Two persons had Vi antibody titers of ≥1:160: one was a presumed chronic carrier, as noted above; the other, with a titer of 1:160, was a 32-year-old female with no previous history of typhoid who had three negative stool cultures. In two households municipal water was collected in tanks which, when tested, contained no chlorine; all other municipal water samples had adequate levels of chorine, and none had significant numbers of fecal coliforms.

Non-typhoidal Salmonella *and* Shigella

Nine (5.8%) of the 155 household members had positive stool cultures for non-typhoidal *Salmonella* (Table 1a), with culture-positive persons identified in seven households. Ages of persons culture-positive for *Salmonella* did not differ significantly from ages of culture-negative persons ($P > 0.05$, Mann-Whitney U test) (Fig. 2a). No significant risk factors for infection were identified when culture-positive household members were matched with culture-negative household members, nor was it possible to show an association between household characteristics (number of persons in household, number of persons per room, water and sanitation facilities) and the presence of culture-positive persons in the household. In the two households with more than one culture-positive person, household members had different species or serotypes of *Salmonella*.

TABLE 1

Species serotype of non-typhoidal Salmonella *and* Shigella *isolated from household members*

Organism	No. symptomatic	No. asymptomatic
a Non-typhoidal *Salmonella*		
agona	0	1
anatum	0	1
panama	2	2
typhimurium	0	1
paratyphi B	0	2
Total	2	7
b *Shigella*		
boydii	0	2
flexneri	4	2
dysenteriae	0	1
Total	4	5

Nine household members (5.8%) had positive cultures for *Shigella* (Table 1b), with culture-positive persons identified in eight households (including 2 households in which persons culture-positive for *Salmonella* were also present). The ages of persons culture-positive for *Shigella* did not differ significantly from ages of culture-negative persons (Fig. 2b). None of the 30 household members <10 years of age were culture-positive. No significant risk factors for infection were identified, either between matched culture-positive and culture-negative household members, or between households with and without culture-positive persons. In the one household that had more than one person culture-positive for *Shigella*, household members were colonized with different species of *Shigella*. All isolates contained the 140 md plasmid associated with invasiveness.[12]

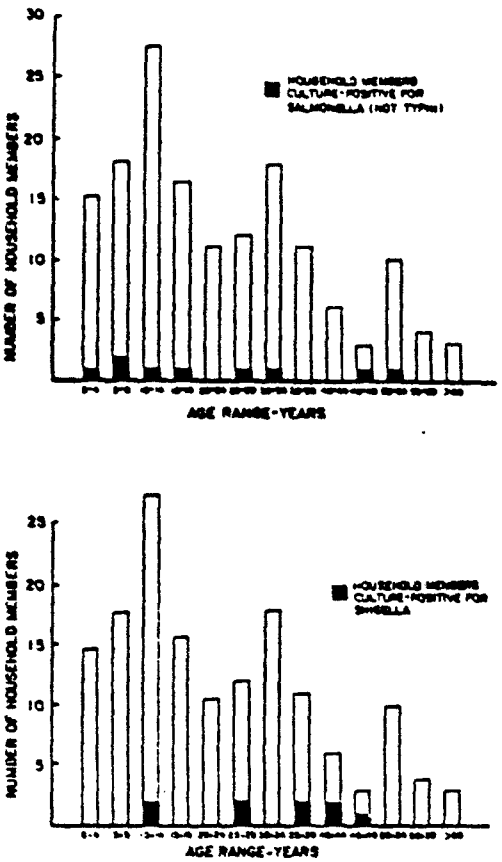

FIGURE 2. a. Ages of all household members, and household members culture-positive for non-typhoidal *Salmonella*. b. Ages of all household members, and household members culture-positive for *Shigella*.

DISCUSSION

In this study we clearly identified only one typhoid chronic carrier among 155 household members, approximately the number which would have been predicted in the general population based on an estimated carrier rate of 694/10^5 persons.[3] These data are in agreement with results of a survey (Cisneros et al., unpublished data) which showed that only two of 81 households of typhoid patients and one of 81 control households had a food handler who was a chronic carrier. While chronic carriers undoubtedly play a role in transmission of the disease in Santiago, these data suggest that acquisition of the disease from carriers within the household accounts for only a small number of the observed cases. We did identify a second person with a borderline elevated Vi antibody titer: the significance of this result is unclear: as the person had three negative stool cultures, this may represent a false-positive test.

We identified possible concurrent or secondary cases in two households. As we did not visit homes until an average of 3 weeks after onset of symptoms in the index case, we may have missed some early cases among household members: in untreated infections, however, stool cultures are most likely to be positive at 3 weeks, with stool carriage continuing until the 7th or 8th week in over 50% of patients.[14] Given the number of young (and potentially susceptible) children in the households studied, our inability to identify additional cases suggests that transmission of *S. typhi* within households (including acquisition by consumption of a common vehicle within a household) was not a frequent occurrence.

We were unable to implicate any one specific vehicle (such as raw vegetables) in the transmission of typhoid. There was an association between age and eating food outside of the household, and a possible interrelationship between these factors and illness: index cases were more likely to eat food outside of the household than were household controls (culture-negative household members < 16 years of age), but cases were also older than household controls, and it was possible to show that older children were more likely than younger children to eat away from home. Typhoid cases in Santiago occur more frequently in older children, with very few cases in children ≤4: if eating food outside of the household is a risk factor for illness, it may partially explain the observed age distribution of cases in the city.

We found a relatively high rate of carriage of non-typhoidal *Salmonella* and *Shigella*, with 11% of household members, in 54% of the households studied, infected with at least one pathogen other than *S. typhi*. In the absence of data from control families it is difficult to know how accurately this reflects the carriage rate of such pathogens in the general population; within the study population, however, the rates are comparable to those seen in countries such as Bangladesh.[15, 16] No household in the study had more than one person culture-positive for the same species of either *Salmonella* or *Shigella*, suggesting, as with *S. typhi*, that these pathogens were infrequently transmitted within households: one might also have

expected to see more cases among young children if household contacts were important in transmission.

With the relatively small size of our study we were not able to identify any specific risk factors for transmission of *S. typhi*, non-typhoidal *Salmonella* or *Shigella*. There did, however, appear to be a relatively high level of exposure to bacterial enteric pathogens within the study population, with transmission or exposure, or both, apparently occurring outside of the immediate household; further studies of the epidemiology of endemic typhoid fever in Santiago should be focused on events of transmission outside the household.

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APPENDIX D

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Benign bacteremia caused by Salmonella typhi and paratyphi in children younger than 2 years

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TYPHOID FEVER HAS REMAINED ENDEMIC in Santiago, Chile, for decades; since 1977 the incidence has exceeded 150 cases per 100,000 population. Typhoid fever occurs mainly in persons 5 to 25 years of age (Table I), and is generally manifested as a classic clinical syndrome including fever, abdominal discomfort and distention, headache, malaise, constipation, and hepatosplenomegaly.

Few cases of typhoid fever are reported in children younger than 2 years. Thus it was necessary to determine whether the very low reported incidence of typhoid fever in young children represents a lack of consumption of the vehicles that transmit *Salmonella typhi* to older children or whether infection occurs but the infant host manifests an atypical response that is not readily recognized clinically. To help resolve this question, we systematically performed blood cultures in children younger than 2 years with fever who were seen at two health centers in Santiago during the 3 peak months of the typhoid fever season.

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METHODS

Rectal temperatures were recorded in all children younger than 2 years who were seen at Pincoya and Consultorio Dos health centers in the northern administrative area (Area Norte) of Santiago from January through March 1983. In all children with a temperature ≥38° C, 2 ml blood was drawn for culture and inoculated into a flask containing 35 ml brain-heart infusion with 0.01% sodium polyanethol sulfonate. The reason for the blood culture was explained to the parents, and verbal informed consent was obtained, according to local custom. The study was discontinued in the last week of March, by which time blood from 197 consecutive children had been cultured. Cultures were

Table I. Age-specific incidence and cases of typhoid and paratyphoid fever, Santiago, Chile, 1977-1981

Metropolitan Santiago*			Northern Administrative Area of Santiago (Area Norte)†		
Age (yr)	Mean annual cases‡	Mean annual incidence per 100,000	Age (yr)	Mean annual cases‡	Mean annual incidence per 100,000
0 to 4	421	89.2	<2	-	27.2
5 to 9	1193	272.2	2 to 4	49	112.6
10 to 14	1413	333.0	5 to 9	149	238.3
15 to 19	1465	283.4	10 to 14	133	305.4
20 to 24	1128	246.7	15 to 19	103	173.4
25 to 34	1023	153.3			
35 to 44	366	74.6			
45 to 54	179	50.2			
55 to 64	86	36.0			
≥65	79	38.5			

*Mean population [illegible]
†Mean population 512,453
‡Approximately 90% of cases in all age groups are typhoid fever, 10% paratyphoid fever

Table II. Clinical findings in infants* with *S. typhi* and *S. paratyphi* bacteremia

Age (mo)	Sex	Isolate	Temperature (°C)	Duration of fever (days)	Anorexia	Vomiting	Constipation	Diarrhea	Cough	Hepatomegaly	Splenomegaly	Clinical diagnosis
4	M	S. typhi	38.4	1	-	+	-	-	-	-	-	Viral syndrome
9	F	S. typhi	38.8	1	-	-	-	-	+	-	-	Viral syndrome
10	M	S. typhi	39.3	1	+	+	-	-	-	-	-	Pneumonitis
17	F	S. typhi	38.3	4	+	-	-	-	-	-	-	Viral syndrome
3	M	S. paratyphi B	38.3	5	-	+	-	-	-	-	-	Bronchopneumonia
9	F	S. paratyphi A	38.4	1	+	-	-	+	+	-	-	Acute bronchitis
14	F	S. paratyphi B	38.4	3	+	-	+	-	-	-	-	Viral syndrome

*Includes only the seven infants detected by active surveillance in this prospective study.

incubated at 35° C for 7 days, and suspicious colonies were confirmed as *S. typhi* by standard biochemical and serologic techniques. *S. typhi* were phage typed at the Institute of Public Health, Santiago. A standardized medical history and physical examination were recorded for all infants. Infants with positive cultures were recalled, reexamined, and given chloramphenicol (50 mg/kg/day PO).

RESULTS

Of the 197 children, 50 were younger than 6 months (no newborn infants), 68 were 6 to 11 months of age, 57 were 12 to 17 months, and 22 were 18 to 23 months; 93% of the fevers recorded were between 38° and 39° C. Acute respiratory infections (44%), diarrhea (20%), and viral syndrome (13%) were the most common clinical diagnoses at the time of examination. None of the infants appeared severely ill, and in no instance was enteric fever considered in the differential diagnosis; consequently, were it not for the study protocol, a blood culture would not have been taken from any infant.

S. typhi was isolated from four children (2%), *S. paratyphi* B from 2 (1%), and *S. paratyphi* A from 1 (0.5%); all other blood cultures were negative. Four isolations occurred in January, one in February, and two in March. Two *S. typhi* strains were nontypable. However, the remaining two were phage type E1 and 46, the two most common types in Santiago.

The clinical syndrome in these infants prior to examination was mild, consisting of 1 to 5 days of fever between 38.3° and 38.8° C (Table II). Six of the seven infants, including all four with *S. typhi*, had cough, and one had clinical and radiologic evidence of pneumonitis. None had splenomegaly, but one had minimal hepatomegaly. On follow-up it was found that none of the infants had completed the course of chloramphenicol therapy; the mothers had spontaneously discontinued the medication

after 1 or 2 days because the infants appeared well. Nevertheless, in each instance the infection resolved without complications.

DISCUSSION

Most information on the age distribution of typhoid fever stems from hospital-based studies.[1-4] Three major points recur in these reports: (1) *S. typhi* infection is notably less common in children younger than 2 years (usually <10% of the cases). (2) The clinical syndrome is often distinct from that encountered in older children, and commonly includes vomiting, diarrhea, convulsions and meningismus, and respiratory signs, in addition to fever. (3) Most reports state that hospitalized infants with typhoid fever are quite ill and that a bacteremic infectious process (e.g., sepsis, meningitis) is usually suspected.

Two main hypotheses have been put forth to explain the low reported incidence of typhoid fever in children younger than 2 years: (1) Infants and young children do not ingest the vehicles of transmission of *S. typhi* that are consumed by older children. (2) Infants and young children consume contaminated vehicles of transmission but do not readily develop recognizable clinical illness because of host factors peculiar to the age group. If the latter is correct, and infants are becoming infected but are manifesting only mild illness, evidence of such infections would have to be sought by systematic investigation of nonhospitalized, mildly ill infants. This pilot study in Santiago, an area where typhoid fever is endemic, represents the first systematic attempt to decipher this problem. The isolation of *S. typhi* and *S. paratyphi* from blood cultures of 3.6% of 197 febrile but mildly ill infants seen at health centers during the summer months demonstrates that during the peak typhoid fever season, children younger than 2 years are becoming infected at a much higher rate than previously appreciated. During this same 3-month period, two infants from the registered population served by these two National Health Service community health centers were admitted directly to the hospital with severe illness confirmed by blood culture to be typhoid fever. Thus at least two mild, unrecognized, bacteremic *S. typhi* infections in young children may exist for every clinically overt, confirmed case.

In the pathogenesis of typhoid fever, two bacteremias occur at distinct stages. The primary bacteremia appears within hours after ingestion of the pathogen.[5] On reaching the small intestine, the *S. typhi* rapidly pass through the mucosa to reach the lamina propria, where they elicit a chemotactic response resulting in an influx of macrophages. Primary access to the bloodstream occurs either during mucosal invasion or after drainage to mesenteric lymph nodes and entrance into the blood by way of the thoracic duct. This primary bacteremia is short-lived and clinically inapparent. Viable *S. typhi* persist in the reticuloendothelial system after being cleared from the blood. After incubation of 10 to 14 days, and concomitant with the onset of clinical illness, the secondary bacteremia characteristic of typhoid fever occurs. It is not clear whether the enteric fever organisms in the blood of these infants represent the fortuitous detection of primary bacteremia or whether it denotes secondary bacteremia in infants with a particularly benign form of the disease. Earlier reports noted the mildness of pathologic alterations caused by *S. typhi* in the intestines of infants[1,3,4,6] compared with those in older children, as well as the frequency of respiratory signs and symptoms.[1,3]

Ashcroft[10] pondered why some less developed areas with appalling sanitation have little typhoid fever, whereas other somewhat more developed countries have endemic disease with high incidences in schoolchildren and young adults. He hypothesized that in areas with the most primitive sanitation and hygiene, widespread asymptomatic or mild infection of infants and young children occurs, leading to immunity and exhaustion of susceptible individuals after the first few years of life. According to Ashcroft's hypothesis, frequent infection of infants and young children would not be expected in a more developed country such as Chile, where the epidemiologic pattern of typhoid reveals the peak reported incidence in schoolchildren and young adults. Nevertheless, our preliminary data support the concept that infants become infected at a higher rate than is commonly appreciated and manifest a very mild clinical illness (not recognized as enteric fever), albeit accompanied by demonstrable bacteremia.

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INVESTIGACION SOBRE EL ESTADO DE PORTADOR DE *SALMONELLA TYPHI-PARATYPHI* EN PACIENTES INTERVENIDOS POR PATOLOGIA VESICULAR[1]

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La elevada morbilidad de la fiebre tifoidea en Chile, junto a los datos demostrativos de que existe una correlación entre el estado de portador de S. typhi *y las colecistopatías encontradas en otros lugares, condujo a analizar 1 000 muestras de bilis de pacientes con colecistopatía. Los resultados indican que la prevalencia excepcionalmente elevada de colecistopatías en Chile constituye un factor importante en la transmisión de la fiebre tifoidea.*

Introducción

En Chile, la morbilidad por fiebre tifoidea presenta una tendencia ascendente, intensificada en forma notable a partir del último quinquenio, con tasas superiores a 120 por 100 000 habitantes (*1*). Esta situación resulta sorprendente, si se considera que el país no se encuentra entre los de menor desarrollo económico o de peores condiciones sanitarias. Por otra parte, el fenómeno se acentúa en Santiago que, si bien dispone de la más alta cobertura del país en agua potable y sistemas para eliminación de excretas, contribuye con dos tercios al total de casos (*1-3*).

Es bien conocido que el papel más importante en la transmisión de la enfermedad lo desempeñan los portadores (*4-6*), cuyo número se incrementa por la existencia de por lo menos tres casos subclínicos o inaparentes por cada caso diagnosticado. Por este motivo, la elevada prevalencia de las colecistopatías en el país (*7-11*) nos indujo a investigar la relación entre esta enfermedad y la transmisión de la fiebre tifoidea, ya mencionada en trabajos realizados en otros países. (Estudios epidemiológicos y anatomopatológicos en Chile han revelado que entre los adultos la litiasis vesicular se observa en una proporción del 50% en el sexo femenino y del 20,5% en el masculino (*7,8*.)

Propósito del estudio

El objetivo del estudio fue analizar la flora microbiana aerobia en la bilis de enfermos sometidos a colecistectomía en la ciudad de Santiago, y en especial las salmonelas del grupo tífico-paratífico. En trabajos similares realizados en países con baja inci-

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dencia de infecciones entéricas, se ha señalado la presencia en la bilis de una gran variedad de bacterias en un tercio de los casos de colecistopatías intervenidas quirúrgicamente, pero sin participación de las salmonelas del grupo tífico-paratífico (*12-18*). Al proyectarse sobre el total de colecistopatías estimado para Santiago, el resultado de esta investigación permitiría disponer de una orientación aproximada con respecto al número de portadores de fiebre tifoidea en la capital.

Material y método

La investigación se realizó con exclusividad en el área urbana de Santiago con la colaboración de siete servicios quirúrgicos de los principales hospitales de adultos. Se tomaron muestras de bilis durante tres meses, a partir de julio de 1980, de pacientes seleccionados al azar, hasta lograr el total preestablecido como meta de 1 000 muestras.

Durante las colecistectomías los cirujanos extrajeron bilis por punción vesicular de los pacientes y todas las muestras se remitieron al Instituto de Salud Pública de Chile, junto con suero sanguíneo de los enfermos. En el Instituto se investigó la presencia de microorganismos aerobios en las muestras mediante cultivos en agar sangre y en medios selectivos salmonella-shigella y desoxicolato xilosa-lactosa para salmonelas, y las muestras de suero sanguíneo se sometieron a la prueba de Widal para detectar la presencia de *S. typhi*. Al mismo tiempo, uno de los hospitales colaboradores (el Hospital San Juan de Dios) realizó una investigación de microorganismos anaerobios en la bilis de sus propios pacientes, cuyos resultados se publicarán por separado.

Resultados

En el cuadro 1 se presenta la distribución por sexo y edad de los 1 000 pacientes con colecistectomía de los cuales se obtuvieron muestras de bilis. La frecuencia de colecistectomías fue mayor en el sexo femenino, con una relación de 4:1 ó 5:1 mujeres por hombre en todos los hospitales, excepto en la Clínica Central que sólo atiende emergencias, donde la relación fue de 2:1 aproximadamente. Este resultado podría explicarse por el número más elevado de intervenciones en hombres, a causa de procesos agudos (empiemas, colangitis y cuadros obstructivos).

Como se indica también en el cuadro 1 la proporción de hombres sometidos a esta operación aumentó con la edad: fue mínima en los menores de 25 años (6,4%) y máxima (32,8%) en los mayores de 55 años. En las mujeres la distribución fue más uniforme, 18,1% en el grupo de menores de 25 años y 19,5% en las que tenían más de 54 años.

Entre las causas de la intervención predominó la litiasis vesicular, en 45,7% de los hombres y 51,9% de las mujeres, seguida por la colecistitis crónica, en 28,6 y 31,2% respectivamente, y por la colecistitis aguda, en 23,0 y 13,5%.

El empiema vesicular, el cáncer y otros diagnósticos sólo participaron en una proporción muy pequeña de las intervenciones (menos de 4%). Sin embargo, el porcentaje de casos de litiasis vesicular fue algo mayor que el indicado ya que un número importante de pacientes operados por colecistitis presentaba litiasis vesicular.

En el cuadro 2 se indica la proporción de bilicultivos que dieron resultados bacteriológicos positivos, según el lugar de intervención y el sexo; uno de los porcentajes más altos de positividad correspondió al Hospital San Juan de Dios, lo que podría deberse a circunstancias especiales en relación con algunas de las muestras. En dicho hospital, además de hacer una investigación de microbios anaerobios, se investigaron también las bacterias aerobias. Estos exámenes no fueron exactamente los mismos que los que se realizaron en el Instituto

CUADRO 1— Pacientes intervenidos que proporcionaron muestras de bilis, según sexo y edad, y hospitales o clínicas de Santiago donde se realizó la operación (julio-agosto 1980).

Hospital o clínica que proporcionó muestras de bilis	Hombres, según grupos de edad (en años)						Mujeres, según grupos de edad (en años)						Total ambos sexos
	<25	25-34	35-44	45-54	≥55	Total	<25	25-34	35-44	45-54	≥55	Total	
San Juan de Dios	1	8	11	7	10	37	29	34	43	17	30	153	190
Barros Luco-Trudeau	2	9	6	9	11	37	26	33	35	21	29	144	181
Salvador	4	9	9	7	6	35	22	40	32	22	36	152	187
San José	1	2	2	5	16	26	17	17	26	11	20	91	117
J.J. Aguirre	3	0	0	2	8	13	13	14	11	8	13	59	72
Sótero del Río	0	5	9	5	9	28	28	56	36	20	16	136	164
Clínica Central de Asistencia Pública	2	5	7	7	7	28	10	11	12	17	11	61	89
Total	13	38	44	42	67	204	145	185	195	116	155	796	1 000
Porcentaje de hombres y mujeres que proporcionaron muestras de bilis	6,4	18,6	21,6	20,6	32,8	100,0	18,2	23,2	24,5	14,6	19,5	100,0	

de Salud Pública, ya que en éste se utilizaron medios selectivos para *S. typhi*, los cuales tal vez limitaron el desarrollo de otras especies. Por lo tanto los resultados de los 51 exámenes realizados en el laboratorio del Hospital San Juan de Dios, que se incluyen en este estudio, quizá contribuyeran al elevado índice de positividad que se encontró en las muestras tomadas en esa institución.

En la Clínica Central de la Asistencia Pública, donde sólo se practican intervenciones de emergencia, con una proporción alta de procesos agudos, se observó el mayor porcentaje de resultados positivos.

Al considerar en conjunto las muestras que se tomaron en los siete establecimientos, la proporción de bilicultivos positivos fue superior en hombres (35,8%) que en mujeres (26,6%), lo que puede relacionarse con la mayor frecuencia de cuadros agudos entre los primeros.

En el cuadro 3 se comparan los resulta-

CUADRO 2— Resultados de los bilicultivos, según sexo y hospitales de Santiago que proporcionaron muestras de bilis.

Hospital o clínica que proporcionó muestras de bilis	Bilicultivos de hombres				Bilicultivos de mujeres				Bilicultivos de ambos sexos			
	Total muestras	No. negativos	No. positivos	% positivos	Total muestras	No. negativos	No. positivos	% positivos	Total muestras	No. negativos	No. positivos	% positivos
San Juan de Dios	37	16	21	56,8	153	115	38	24,8	190	131	59	31,1
Barros Luco-Trudeau	37	30	7	18,9	144	101	43	29,9	181	131	50	27,6
Salvador	35	23	12	34,3	152	116	36	23,7	187	139	48	25,7
San José	26	21	5	19,2	91	75	16	17,6	117	96	21	17,9
J.J. Aguirre	13	9	4	30,8	59	40	19	32,2	72	49	23	31,9
Sótero del Río	28	18	10	35,7	136	101	35	25,7	164	119	45	27,4
Clínica Central de Asistencia Pública	28	14	14	50,0	61	36	25	41,0	89	50	39	43,8
Total	204	131	73	35,8	796	584	212	26,6	1 000	715	285	28,5

dos obtenidos en hombres y mujeres de diferentes grupos de edad. En los hombres, si no se toma en consideración el pequeño número de muestras de los menores de 25 años, se observa un aumento de positividad en los grupos de mayor edad. Este aumento no fue tan pronunciado en las mujeres, con excepción del grupo mayor de 55 años en donde el porcentaje de positividad fue 49,4.

Como se indica en el cuadro 4, de los 1 000 bilicultivos examinados se aislaron 340 bacterias, lo que coincide con los resultados de investigaciones realizadas en otros países. *Escherichia coli* fue la bacteria que se encontró con mayor frecuencia (con 33,5% del total de muestras positivas) seguida por las del grupo tífico-paratífico (21,5%), *Klebsiella pneumoniae* (11,8%) y *Streptococcus viridans* (6,5%). Las demás bacterias aerobias se encontraron en proporciones menores. En total se aislaron 340 bacterias de 285 bilicultivos, ya que en algunos de ellos coexistían varias bacterias.

En el cuadro 5 se muestra la distribución de salmonelas, *E. coli* y otras bacterias, según el sexo y la edad de los pacientes. En general, se halló un mayor porcentaje de *E. coli* en bilicultivos positivos de hombres (18,1%) que de mujeres (9,7%). Su proporción aumentó con la edad en ambos sexos y resultó máxima en el grupo mayor de 54 años. A menudo se aisló *E. coli* en bilicultivos que contenían también otras bacterias. La proporción de muestras de bilis positivas para *S. typhi* y *S. paratyphi* fue muy similar. *S. typhi* se encontró en el 2,9% de los bilicultivos del sexo femenino y 4% de los del sexo masculino, y *S. paratyphi* en el 2,5 y 3,1% respectivamente. En cuanto a la distribución de estas variedades según la edad de los pacientes, no pudo analizarse eficazmente en los hombres debido al reducido número de muestras. En las mujeres se observó una leve disminución de los porcentajes de cultivos positivos al aumentar la edad, pero no fue estadísticamente significativa. Como *S. typhi* es responsable de la mayoría de las infecciones entéricas causadas también por *S. paratyphi* es evidente que los resultados (que muestran porcentajes aproximadamente iguales de los dos microorganismos) no reflejen lo observado en la clínica.

CUADRO 3— Comparación de los resultados obtenidos en bilicultivos de pacientes de ambos sexos, según la edad.

Sexo de los pacientes que proporcionaron muestras de bilis y resultados de los bilicultivos	Edad de los pacientes que proporcionaron muestras (en años)										Todos los grupos de edad	
	<25		25-34		35-44		45-54		≥55			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Muestras de hombres:												
Positivo	6	42,9	6	16,2	16	36,4	17	40,5	28	41,8	73	35,8
Negativo	8	57,1	31	83,8	28	63,6	25	59,5	39	58,2	131	64,2
Total	14	100,0	37	100,0	44	100,0	42	100,0	67	100,0	204	100,0
Muestras de mujeres												
Positivo	31	21,5	33	17,8	40	20,5	31	26,7	77	49,4	212	26,6
Negativo	113	78,5	152	82,2	155	79,5	85	73,3	79	50,6	584	73,4
Total	144	100,0	185	100,0	195	100,0	116	100,0	156	100,0	796	100,0
Todas las muestras:												
Positivo	37	23,4	39	17,6	56	23,4	48	30,4	105	47,1	285	28,5
Negativo	121	76,6	183	82,4	183	76,6	110	69,6	118	52,9	715	71,5
Total	158	100,0	222	100,0	239	100,0	158	100,0	223	100,0	1 000	100,0

CUADRO 3.— Bacterias aisladas de muestras de bilis de 1 000 pacientes.

Bacterias aisladas	Hospital o clínica que proporcionó las muestras							Total (todos los hospitales)	
	Clínica Central de Asistencia Pública	Barros Luco-Trudeau	J.J. Aguirre	Salvador	Sra. José	San Juan de Dios	Sótero del Río	No.	%
Salmonella typhi	5	5	2	6	2	10	8	38	11,2
Salmonella paratyphi A	—	2	—	—	2	1	—	5	1,5
Salmonella paratyphi B	5	3	2	6	2	4	8	30	8,8
Escherichia coli	14	23	5	15	9	37	11	114	33,5
Klebsiella pneumoniae	4	4	4	6	2	13	7	40	11,8
Aerobacter hidrophyla	—	—	—	—	—	2	—	2	0,6
Enterobacter aerog.	—	1	—	—	—	1	—	2	0,6
Enterobacter agglom.	1	3	—	3	1	—	2	10	2,9
Enterobacter hafn.	2	—	1	1	—	—	5	9	2,6
Serratia marcescens	—	—	—	—	—	—	1	1	0,3
Citrobacter freundii	1	3	1	2	—	3	—	12	3,5
Citrobacter diversus	—	—	—	—	1	—	—	1	0,3
Alcalescens dispar	—	—	—	1	1	—	—	2	0,6
Proteus mirabilis	3	3	—	—	1	6	—	13	3,8
Proteus vulgaris	—	—	—	—	—	1	—	1	0,3
Proteus morganii	3	—	—	1	—	—	—	4	1,2
Pseudomonas aeruginosa	—	1	1	—	—	1	1	4	1,2
Pseudomonas stutzeri	—	—	1	1	—	—	—	2	0,6
Staphylococcus aureus	1	—	—	3	—	2	—	6	1,8
Staphylococcus epidermidis	2	1	1	—	—	1	—	5	1,5
Streptococcus viridans	1	2	3	3	3	7	3	22	6,5
Enterococos	—	—	—	—	—	9	—	9	2,6
Anaerobios	—	—	—	—	—	8	—	8	2,4
Total de bacterias	42	53	21	48	24	106	46	340	100,0
Total de muestras	89	179	72	189	117	190	164	1 000	
% de bacterias aisladas en el total de muestras examinadas	47,2	29,6	29,2	25,4	20,5	55,8	28,0	34,0	

En cuanto al tiempo transcurrido entre la obtención y el análisis de las muestras de bilis, el cuadro 6 indica que las muestras procesadas 72 horas después de la colecistectomía resultaron positivas en 30,4%, y disminuyeron a 24% cuando el plazo fue mayor. (Todas las muestras se procesaron dentro de los siete días después de su recolección.) En general, en las bacterias del grupo tífico-paratífico se halló una positividad de 7,7% cuando el tiempo transcurrido antes de la siembra fue menor de 72 horas, con una disminución a 6,6% en un lapso mayor. Los otros tipos de bacterias resultaron aún más afectados al aumentar el tiempo transcurrido, con 22,7% de positividad en lapsos menores de 72 horas y 17,4% en los mayores de ese límite.

En el cuadro 7 se comparan los resultados de los bilicultivos y las reacciones de aglutinación (Widal), obtenidas con las muestras de sangre de los pacientes. Como puede observarse hubo un aumento de positividad del cultivo a medida que se elevaban los títulos de anticuerpos H y O, detectados por la reacción de Widal. Sólo 1,6% de los enfermos con reacción negativa para el antígeno H (con títulos menores de 1:4

CUADRO 8—*E. coli*, *S. typhi*, *S. paratyphi* A, *S. paratyphi* B y otras bacterias aisladas en 1 000 bilicultivos examinados, según sexo y edad de los pacientes.

No. de bilicultivos examinados y resultados	Bilicultivos de hombres según edad de los pacientes (en años)[1]										Bilicultivos de mujeres, según edad de las pacientes (en años)										Todos los bilicultivos	
	<25		25-34		35-44		≥55		Total		<25		25-34		35-44		≥55		Total			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total de muestras obtenidas	14	100,0	37	100,0	44	100,0	42	100,0	204	100,0	144	100,0	185	100,0	195	100,0	156	100,0	796	100,0	1 000	100,0
Resultados negativos	8	57,1	31	83,8	28	63,6	25	59,5	59	64,2	113	78,5	152	82,2	155	79,5	85	73,3	29	50,6	581	73,0	715	71,5
Resultados positivos para:																						
E. coli	2	14,3	4	10,8	8	18,2	8	19,0	15	22,4	37	18,1	5	3,5	11	5,9	12	6,2	16	13,8	33	21,2	77	9,7	114	11,4
S. typhi	1	7,1	0	0,0	5	11,4	0	0,0	0	0,0	6	2,9	0	5,6	0	4,1	5	2,6	5	4,3	6	3,8	32	4,0	38	3,8
S. paratyphi A	1	7,1	0	0,0	0	0,0	1	2,4	0	0,0	2	1,0	0	0,0	2	1,1	0	0,0	0	0,0	1	0,6	3	0,4	5	0,5
S. paratyphi B	0	0,0	1	2,7	1	2,3	2	4,8	1	1,5	5	2,5	8	5,6	3	1,6	4	2,1	4	3,4	6	3,8	25	3,1	30	3,0
Otras bacterias	2	14,3	3	13,5	8	18,2	8	19,0	16	23,9	30	18,6	31	21,5	33	17,8	40	20,5	31	26,7	77	49,4	212	26,6	285	28,5

y 1,7% en el caso del antígeno O, tuvieron muestras de bilis positivas para *S. typhi*. De manera contraria, sólo 181 (19,9%) de los pacientes con muestras negativas para *S. typhi* tuvieron reacciones positivas frente al antígeno H, y únicamente 122 (13,4%) fueron positivos frente al anticuerpo O.

En el cuadro 8 se comparan los resultados de los bilicultivos y los antecedentes de enfermedad de los pacientes con respecto a una infección entérica previa. En hombres, los cultivos positivos para *S. typhi* coincidieron con un antecedente de enfermedad entérica en 20,8%, mientras que sólo 4,4% sin antecedentes o recuerdo de infección, tuvieron cultivos positivos para ambos tipos de bacterias. De manera similar, se obtuvieron cultivos positivos en 20,4% de las mujeres con antecedentes de enfermedad entérica (en comparación con 5,7% sin recuerdo de infección). Como es obvio, el antecedente se precisó mejor en casos de infecciones recientes. Por otra parte, cabe señalar que se hubieran obtenido resultados más definidos en favor del paralelismo entre el antecedente y la positividad del cultivo al no mediar la frecuencia de casos subclínicos o ambulatorios, donde la infección no era evidente.

Discusión y conclusiones

En Chile, la incidencia anual de la fiebre tifoidea supera a la de países con menor desarrollo económico y condición climática más favorable para la transmisión de enfermedades. El hecho es aún más notorio en la ciudad de Santiago, donde se registran dos tercios del total de casos con sólo un tercio de la población total del país.

Se ha comprobado que más que los enfermos, los portadores de *S. typhi* juegan un papel preponderante en la transmisión de la enfermedad, pero los estudios destinados a demostrar este hecho se han basado siempre en el coprocultivo, método impreciso y resistido por los pacientes si se repite en forma seriada.

CUADRO 6— Efecto del tiempo transcurrido entre la obtención de la muestra y la siembra, sobre el aislamiento de bacterias.

Resultados bacteriológicos	Tiempo transcurrido entre la obtención de la muestra y la siembra					
	≤72 horas		>72 horas			
	No. de muestras	%	No. de muestras	%	Se ignora	Todas las muestras
Resultados negativos	487	68,6	127	76,0	91	715
Resultados positivos para:						
S. typhi-paratyphi	55	7,7	11	6,6	7	73
Otras bacterias	162	22,7	29	17,4	21	212
Total de muestras	714	100,0	167	100,0	119	1 000

Además, existen datos de que las bacterias del grupo tífico-paratífico son causa de colecistopatías y que si infectan a personas que ya padecen de procesos vesiculares, la persistencia del estado de portador es más frecuente y prolongada que en las personas que no padecen colecistopatía. A lo anterior debe añadirse que la prevalencia de colecistopatías en Chile es una de las más elevadas del mundo.

Otro dato significativo es que durante el estudio se practicaron cuatro veces más colecistopatías en mujeres que en hombres. En general, éstos tuvieron intervenciones por procesos agudos, mientras que en la mayoría de las mujeres predominó el diagnóstico de colecistitis crónica o litiasis vesicular.

En lo que se refiere a los resultados de los bilicultivos, sólo *E. coli*, presente en 33,5% de las muestras positivas, superó en frecuencia al grupo *S. typhi-paratyphi*, que se halló en 21,5%, así como a cualquier otra magnitud registrada en la bibliografía. Al respecto, conviene notar que los informes anteriores sobre este tema corresponden a países con mayor desarrollo económico y baja incidencia de infecciones entéricas. La repetición de este tipo de investigaciones en

CUADRO 7— Relación entre los pacientes con reacciones de aglutinación positivas (Widal) para anticuerpos H y O (títulos 1:40 o más altos) y los pacientes con muestras de bilis positivas para *S. typhi*.

	Resultados bacteriológicos de bilicultivos					
	Positivo para *S. typhi*		Negativo para *S. typhi*		Total de bilicultivos	
	No.	%	No.	%	No.	%
Títulos obtenidos con antígeno H:						
≤1:20	12	1,6	728	98,4	740	100
1:40 ó 1:80	15	8,3	166	91,7	181	100
≥1:160	8	34,8	15	65,2	23	100
Títulos obtenidos con antígeno O:						
≤1:20	14	1,7	787	98,3	801	100
1:40 ó 1:80	15	11,4	117	88,6	132	100
≥1:160	6	54,5	5	45,5	11	100
No. de pacientes examinados	35		909		944	
No. de pacientes no examinados	3		53		56	
Total de pacientes	38		962		1 000	

CUADRO 8— Relación entre los resultados de los bilicultivos y los antecedentes de enfermedad de los pacientes.

Antecedentes de enfermedad	Bilicultivos de hombres						Bilicultivos de mujeres						Todos los bilicultivos					
			Positivo para *S. typhi-paratyphi*						Positivo para *S. typhi-paratyphi*						Positivo para *S. typhi-paratyphi*			
			Sí		No				Sí		No				Sí		No	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Con antecedentes de enfermedad entérica	24	100	5	20,8	19	79,2	98	100	20	20,4	78	79,6	122	100	25	20,5	97	79,5
Sin antecedentes de enfermedad entérica	180	100	8	4,4	172	95,6	698	100	40	5,7	658	94,3	878	100	48	5,5	830	94,5
Total	204	100	13	6,4	191	93,6	796	100	60	7,5	736	92,5	1 000	100	73	7,3	927	92,7

países donde la fiebre tifoidea aún constituye un problema grave, otorgaría mayor validez a las conclusiones de este estudio.

No se observó una relación clara entre el aislamiento de bacterias específicas en los bilicultivos y los antecedentes de enfermedad, salvo en los casos de infección muy reciente. Esto puede atribuirse sobre todo a la frecuencia de formas ambulatorias, no advertidas, en especial en el caso de *S. paratyphi* B, cuyo aislamiento de los bilicultivos, casi tan alto como el de *S. typhi*, no guarda relación con la frecuencia mínima de su diagnóstico clínico.

En cambio, hubo cierta concordancia entre la positividad de los cultivos y los títulos de anticuerpos H y O detectados mediante las reacciones de aglutinación de Widal.

La positividad de *S. typhi-paratyphi* (7,3%) obtenida en los bilicultivos examinados, al proyectarse sobre el total de colecistopatías en el área metropolitana de Santiago (500 000) o en todo el país (1 200 000), permite deducir la enorme cantidad de portadores circulantes, sobre todo, del sexo femenino, quienes en su mayoría suelen ocuparse de la manipulación de alimentos.

El riesgo de que los casos diagnosticados y notificados de infecciones por *S. typhi* y *S. paratyphi*, junto con el mayor número de casos subclínicos o inaparentes, se transformen en portadores crónicos se magnifica por las elevadas tasas de colecistopatías y litiasis vesiculares. Se explica así la inusitada incidencia de estas infecciones en un país cuyo nivel socioeconómico y condiciones tanto sanitarias como climáticas no se encuentran entre los más desfavorables del mundo para la transmisión de esos organismos. Las esperanzas de reducir al máximo este problema dependen en gran parte del éxito que se logre en los ensayos de nuevas vacunas vivas y atenuadas de administración oral que, además de proporcionar protección contra las manifestaciones clínicas, sean capaces de producir inmunidad intestinal, con la consecuente reducción del número de portadores.

Resumen

En años recientes la morbilidad de la fiebre tifoidea en Chile ha sido relativamente alta y la incidencia de la enfermedad se ha elevado hasta 120 casos por 100 000 habitantes. Como en estudios realizados en otros países se ha encontrado una relación entre la colecistopatía y el estado de portador, al que puede atribuirse gran parte de la transmisión de la fiebre tifoidea, se realizó un análisis de muestras de bilis y de sangre de

1 000 pacientes intervenidos por colecistopatía, durante el período de julio a octubre de 1980. Los siete hospitales que proporcionaron las muestras se encontraban ubicados en el área metropolitana de Santiago, en donde la incidencia de la fiebre tifoidea era considerablemente más alta que en el resto del país.

Las colecistectomías fueron aproximadamente cuatro veces más frecuentes en las mujeres que en los hombres, lo que confirma el hecho de que la incidencia de colecistopatías es mayor en el sexo femenino. Sin embargo, un porcentaje más alto de hombres ingresaron en los hospitales por colecistitis aguda.

Se encontraron bacterias en el 35,8% de los bilicultivos de pacientes del sexo masculino y en el 28,5% de los del sexo femenino. En los 285 bilicultivos positivos se encontraron 38 *Salmonella typhi* y 35 *S. paratyphi*. En conjunto, sólo se aisló *S. typhi* en el 11,2% de los bilicultivos positivos y en el 3,8% de las 1 000 muestras examinadas. Estos resultados concuerdan bastante bien con los obtenidos mediante las reacciones de aglutinación de Widal que se efectuaron con muestras de sangre de los mismos pacientes.

Las colecistopatías son bastante frecuentes en Chile; sólo en el área de Santiago se ha estimado que existen 500 000 casos. Este hecho, unido a la frecuencia de estados portadores en los casos de colecistopatía, como se deduce de los resultados de este estudio, permite explicar la gran incidencia de la fiebre tifoidea.

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Investigation of the *Salmonella typhi-paratyphi* carrier state in cases of surgical intervention for gallbladder disease (Summary)

Chile has experienced relatively high typhoid morbidity in recent years, the annual incidence going as high as 120 cases per 100 000 inhabitants. Because correlations had been found elsewhere between gallbladder disease and the carrier state responsible for much typhoid transmission, a study was made of bile and blood specimens from 1 000 patients whose gallbladders were surgically removed in July-October 1980. The seven health facilities providing these specimens were located in metropolitan Santiago, which had been experiencing a considerably higher typhoid incidence than the rest of the country.

About four times as many surgical interventions were performed on women than on men, confirming that there was a generally higher incidence of gallbladder disease among the former. However, a higher proportion of the male patients were admitted for acute vesicular disease.

Bile specimens yielding bacterial isolates were obtained from 35,8% of the male patients and 28,5% of the female patients. These 285 positive specimens yielded 38 *Salmonella typhi* and 35 *S. paratyphi* isolates. Overall, *S. typhi* was isolated from 11,2% of the positive bile specimens and 3,8% of the 1 000 specimens examined. These results correlated fairly well with the results of Widal agglutination tests performed with blood specimens from the same patients.

Gallbladder pathologies are quite common in Chile, there being an estimated 500 000 cases in Santiago alone. This fact, together with the frequent occurrence of the carrier state in gallbladder disease cases — as shown by the findings of this study — helps to explain the high observed incidence of typhoid fever.

Pesquisa sobre o estado de portador da *Salmonella typhi-paratyphi* em doentes operados devido à patologia vesicular (Resumo)

Nestes últimos anos a morbilidade da febre tifóide no Chile tem sido relativamente alta e a incidência da doença atingiu até 120 casos por 100 000 habitantes. Da mesma maneira em que estudos feitos em outros países o demonstraram, encontrou-se uma relação entre a colecistopatia e o estado de portador, ao qual se pode atribuir em grande parte a

ransmissão da febre tifóide. Fez-se analise de amostras de bílis e de sangue de 1 000 doentes operados de colecistostomia durante o período de julho a outubro de 1980. Os sete hospitais que forneceram as amostras ficavam na área metropolitana de Santiago, onde a incidência da febre tifóide era notavelmente mais alta do que no resto do país.

As colecistostomias foram aproximadamente quatro vezes mais freqüentes entre as mulheres que entre os homens, o que confirma o fato de que a incidência de colecistopatias seja mais alta no sexo feminino. No entretanto, uma percentagem mais alta de homens baixou aos hospitais devido à colecistite aguda.

Acharam-se bactérias em 35,8% das bilioculturas de doentes do sexo masculino e em 28,5% das do sexo feminino. Nas 285 bilioculturas positivas, acharam-se 38 *Salmonella typhi* e 35 *S. paratyphi*. Em conjunto, somente se isolou *S. typhi* em 11,2% das bilioculturas positivas e em 3,8% das 1 000 amostras examinadas. Esses resultados concordam bastante bem com os que foram obtidos mediante as reações de aglutinação de Widal feitas com amostras de sangue dos próprios doentes.

As colecistopatias são bem freqüentes no Chile. Fez-se uma estimativa que só na área de Santiago há 500 000 casos. Tudo isto acrescentado à freqüência de estados portadores nos casos de colecistopatia, como se pode deduzir dos achados deste estudo, permite explicar a grande incidência da febre tifóide.

Recherche sur l'état de porteur de *Salmonella typhi-paratyphi* chez des patients opérés pour pathologie vésiculaire (Résumé)

Au cours des dernières années la morbilité causée par la fièvre typhoïde au Chili a été relativement importante et l'incidence de cette maladie s'est élevée jusqu'à 120 cas par 100 000 habitants. Etant donné que, lors d'études réalisées dans d'autres pays, on a observé un rapport entre la colecystopathie et le fait d'être porteur, auquel on peut attribuer en grande partie de la transmission de la fièvre typhoïde, une analyse d'échantillons de bile et de sang de 1 000 patients ayant subi une colecystostomie fut effectuée, pendant la période de juillet à octobre 1980. Les sept hôpitaux qui fournirent les échantillons se trouvaient situés dans ou la zone métropolitaine de Santiago, où l'incidence de fièvre typhoïde était considérablement plus élevée que dans le reste du pays.

Les colecystotomies furent approximativement quatre fois plus fréquentes chez les femmes que chez les hommes, ce qui confirme le fait que l'incidence de colecystopathie est plus courante dans le sexe féminin. Cependant, un pourcentage plus élevé d'hommes entrèrent à l'hôpital pour colecystite aiguë.

On observa des bactéries dans 35,8% des bilicultures de patients du sexe masculin et dans 28,5% des bilicultures de malades du sexe féminin. Dans les 285 bilicultures positives on découvrit 38 *Salmonella typhi* et 35 *S. paratyphi*. Pour l'ensemble, on isola *S. typhi* dans 11,2% des bilicultures positives et dans 3,8% des 1 000 échantillons examinés. Ces résultats concordent assez bien avec ceux obtenus par les réactions d'agglutination de Widal qui furent effectuées avec des échantillons de sang des mêmes patients.

Les colecystopathies sont assez fréquentes au Chili; on a estimé leur nombre à 500 000 cas pour la seule région de Santiago. Ce fait, uni au nombre de porteurs dans les cas de colecystopathie, comme on peut le déduire des résultats de cette étude, permet d'expliquer la grande incidence de la fièvre typhoïde.

APPENDII

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Precise Estimation of the Numbers of Chronic Carriers of *Salmonella typhi* in Santiago, Chile, an Endemic Area

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As part of a program to control endemic typhoid fever in Santiago, Chile, an assessment was made of the magnitude of the reservoir of chronic carriers of *Salmonella typhi*. The availability of an accurate census and reliable data on the prevalence of biliary disease and of *S. typhi* carriage among persons with cholecystitis allowed an unusually precise estimate of the number of carriers. In 1980 there existed 25,019 female and 4,575 male carriers in a population of 4,264,514, yielding a crude prevalence of 694 carriers per 10^5 population. Because of the magnitude of this human reservoir, which includes many females of <40 years of age, it is recommended that a typhoid control program include the identification of carriers followed by health education and therapeutic interventions.

The human population is the reservoir as well as the natural host for *Salmonella typhi*. In general, ~2%-5% of all individuals who develop clinical or subclinical infection with *S. typhi* become chronic gallbladder carriers and thereby serve to maintain endemicity of the disease [1-6]. The propensity to become a chronic carrier after acute infection increases with age and is greater in women [1, 2, 5, 7], observations which are in keeping with the epidemiology of cholelithiasis [8-11].

Typhoid fever is highly endemic in Santiago, Chile, despite the widespread availability of potable water, the sewered sanitation, and the effective control of most other communicable diseases [12, 13]. Chile also has one of the highest prevalences of cholelithiasis in the world [10, 11, 14]. This combination of a high incidence of typhoid fever and a high prevalence of gallbladder disease probably results in a high prevalence of chronic carriers. Continued contamination of vehicles of transmission by these carriers maintains the endemic cycle and interferes with effective control of typhoid fever.

As part of a program to control endemic typhoid fever in Santiago, we estimated the number of chronic *S. typhi* carriers. The availability in Santiago of a reliable census, coupled with a large necropsy survey of the prevalence of cholelithiasis and quantitative data on the frequency of *S. typhi* carriage among persons with cholecystic disease, provided an opportunity to assess the magnitude of the human reservoir of infection with a precision heretofore not possible.

Materials and Methods

The sizes of the male and female populations of Santiago were obtained from official census data [15]. The prevalence of persons with gallbladder disease in each decennial age group over 10 years of age was obtained from 1,967 autopsies performed at the Medico-Legal Institute, Santiago [10]; in the vast majority of instances, these autopsies were performed on persons who died as a result of motor vehicle accidents or other trauma [10]. The percentage of persons in each age group who had gallbladder disease was multiplied by the number of persons of that age in the general population to estimate the number with gallbladder disease.

The prevalence of chronic infection of the gallbladder with *S. typhi* among persons with biliary disease in 1980 is known from a recent study of persons undergoing cholecystectomy in seven ma-

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Table 1. Estimate of the number of chronic carriers of *Salmonella typhi* in Santiago, Chile, in 1930 based on the prevalence of gallbladder disease in the population and the prevalence of chronic infection with *S. typhi* in persons with cholelithiasis.

Characteristic	Age group (years)								Total
	10–19	20–29	30–39	40–49	50–59	60–69	70–79	≥80	
Female	443,408	407, 190	323,987	221,209	169,221	113,581	59,235	21,714	1,759,548
Cholelithiasis (%)*	9.7	23.4	43.1	51.7	60.0	69.2	69.2	55.5	. . .
Cholelithiasis†	43,010	95,282	139,638	114,365	101,533	78,593	40,993	12,051	620,675
S. typhi carrier‡	1,720	3,811	5,586	4,575	4,061	3,144	1,640	482	25,019
Carriers per 10^5 population	388	940	1,724	2,068	2,400	2,768	2,768	2,220	. . .
Male	438,665	373,895	294,595	192,984	139,772	82,583	39,221	9,536	1,572,551
Cholelithiasis (%)*	0	4.5	13.4	16.7	19.8	24.7	43.5	40.0	. . .
Cholelithiasis†	0	16,825	39,476	32,395	27,675	20,472	17,061	3,814	157,718
S. typhi carrier‡	0	488	1,145	939	803	594	495	111	4,575
Carriers per 10^5 population	0	131	389	484	575	717	1,262	1,164	. . .

NOTE. Data are no. of persons except where percentages are indicated.

* Percentages based on 1,967 persons studied at autopsy at the Medico-Legal Institute, Santiago.

† Estimate computed by multiplying the no. of persons in each age group of the general population by the percentage of persons in that age group who were found to have cholelithiasis at autopsy.

‡ Estimate based on the observation that 4% of females and 2.9% of males with gallbladder disease in Santiago have chronic *S. typhi* bile infection [16].

jor hospitals in Santiago [16]. Cultures of bile and gallbladder were made at the time of surgery from 1,000 consecutive patients of all ages. The mean prevalence of *S. typhi* carriage was 4.0% among the 796 female patients, and there was little variation by age; the mean prevalence of *S. typhi* among the 204 male patients was 2.9%. The mean prevalence of *S. typhi* infection for each sex was multiplied by the calculated number of persons with gallbladder disease within each age group to derive the number of chronic carriers.

Results

A summary of the population of Santiago in 1980 by age and sex, the number of persons with gallbladder disease, and the calculated number of *S. typhi* carriers is shown in table 1. In total, 25,019 female and 4,575 male chronic carriers of *S. typhi* over 10 years of age were calculated to exist among the population of 4,264,514 in greater Santiago; the overall prevalence was 694 carriers per 10^5 population. The prevalence of chronic carriers increased with age; among women over 40 years of age, 2.1%–2.8% (that is, 2,068–2,768 per 10^5 women) were computed to be chronic *S. typhi* carriers.

Discussion

Typhoid fever is highly endemic in Chile, (the annual incidence since 1975 has ranged from 59 to 121 cases per 10^5 population), particularly in Santiago where peak incidence rates occur in older schoolchildren and young adults. This has been somewhat enigmatic to epidemiologists, since based on other demographic, socioeconomic, and health indicators, Chile is a fairly developed country. The relationship between biliary disease and chronic *S. typhi* carriage has been recognized for many decades [1–7]. Chile also has one of the highest prevalences of gallbladder disease in the world [10, 11, 14], and gallbladder disease appears among young female Chileans [10, 11]. We therefore surmised that there must exist a particularly high prevalence of chronic carriers in Santiago who serve as reservoirs and disseminators of *S. typhi* and who help maintain a high level of endemicity of typhoid fever.

The present report provides the most precise estimation ever made of the number and prevalence of chronic carriers of *S. typhi* in an endemic area. This precision was possible because of the existence of accurate data providing the age-specific prevalence of cholelithiasis [10, 11] and the frequency of chronic biliary infection with *S. typhi*

among persons with gallbladder disease [16]. The few previous attempts at estimating the number of chronic carriers of *S. typhi* in other geographic areas were rough estimates which, with one exception, did not take into account the relationship between the age of the patient at the time of acute infection and the development of the chronic carrier state [7, 17, 18].

The large number of chronic carriers of *S. typhi* (29,594 persons) and the high crude prevalence rate (694 carriers per 10^5 population) calculated for Santiago demonstrate the notable magnitude of the human reservoir of *S. typhi*. Furthermore, the existence of many carriers younger than 30 years of age implies that a significant reservoir will be present for many decades to come.

The outstanding efficacy of Ty 21a attenuated *S. typhi* oral vaccine in preventing acute typhoid fever in Alexandria, Egypt, has generated considerable hope that mass application of this vaccine in endemic areas may greatly diminish the incidence of typhoid fever and can serve as the keystone of typhoid fever control programs [19]. Nevertheless, the identification of chronic *S. typhi* carriers followed by health education, counseling, and treatment should also be considered critical components of a typhoid fever control program. In this context it would be particularly beneficial to identify young carriers (<40 years of age) who will play a role for many decades in disseminating *S. typhi*. Simple serologic [20] and bacteriologic [21] methods have become available to screen for *S. typhi* biliary carriers. Similarly, preliminary experience suggests that there now exists an effective, nonsurgical, domiciliary therapy to eradicate chronic *S. typhi* gallbladder infection [22]; the therapy involves a 28-day course of oral amoxicillin and probenecid [22] (C.L., unpublished observations). Identification, supervision, and treatment of chronic carriers should be part of a typhoid fever control program.

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Public Health

VI SEROLOGY IN DETECTION OF CHRONIC SALMONELLA TYPHI CARRIERS IN AN ENDEMIC AREA

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Summary A passive haemagglutination assay measuring antibody to highly purified Vi antigen, known to be sensitive and specific for the detection of chronic *Salmonella typhi* carriers in a non-endemic area, was assessed in an endemic area. A reciprocal serum Vi antibody titre of 160 was found to have a sensitivity of 75%, specificity of 92%, and a high predictive value in screening for chronic *S typhi* carriers in high-risk population groups (eg, women over 40 years). This simple assay can screen for chronic *S typhi* carriers even in areas where typhoid fever is highly endemic.

INTRODUCTION

SINCE the description in the 1930s of the Vi antigen of *Salmonella typhi*[1] and of the relation between high serum titres of Vi antibody and the chronic *S typhi* carrier state,[2] there have been many conflicting reports about the usefulness of Vi serology in the detection of chronic *S typhi* carriers. The disagreements stem from both the methods used and the interpretation of results. Felix and others used a Vi-rich *S typhi* strain as the antigen in a direct bacterial agglutination test, which they considered helpful in identifying chronic *S typhi* carriers. Some authorities placed much confidence in this screening test that they required a negative Vi serology to be documented in all individuals employed in certain industries, such as the water and food industries.[3] However, the usefulness of this test, especially in areas where typhoid fever was endemic, was challenged by others; they pointed out that in such areas up to 20% of bacteriologically confirmed chronic *S typhi* carriers lacked Vi antibody and up to 20% of normal individuals with negative cultures for *S typhi* had positive direct bacterial agglutination tests.[4] When the direct bacterial agglutination test was used in mass screening of unselected populations, less than 2% of people with positive Vi serology were found by intensive bacteriological culturing to be chronic *S typhi* carriers.[3,4]

Immunologically identical Vi antigen was discovered in other Enterobacteriaceae, including *Escherichia coli*, *Citrobacter*, and *S paratyphi C*. Crude or partially purified Vi antigen prepared from *S typhi* Vi 1, *E coli*, or *Citrobacter* species was adsorbed to human group O or sheep erythrocytes for a passive haemagglutination assay. This assay had greater sensitivity than the indirect bacterial agglutination test[5] but the high false-positive rate persisted.[6]

In 1972 Wong and Feeley[7] described a method to prepare highly purified Vi antigen. They and their colleagues[8,9] used the highly purified antigen in a passive haemagglutination assay to detect a small number of chronic *S typhi* carriers in a non-endemic area (Arkansas, USA); Vi serology was positive in all 7 chronic *S typhi* carriers tested but in only 1 of 37 (3%) of the stool-culture-negative contacts of these carriers. To determine whether this test might be useful in an endemic area, we have evaluated the sensitivity and specificity of the passive haemagglutination assay with highly purified Vi antigen to detect chronic *S typhi* carriers in Santiago, Chile, where there are an estimated 28 000 or more chronic *S typhi* carriers[10] and where typhoid fever is still an important public health problem.

SUBJECTS AND METHODS

We defined a chronic *S typhi* carrier as an individual from whom *S typhi* was isolated 1 or more years after a bacteriologically confirmed episode of typhoid fever.[11] Since there is a high prevalence of biliary carriage of *S typhi* among Chilean women,[12] we identified from medical records of the Infectious Diseases Hospital, Santiago, women 25 years and older who had had bacteriologically confirmed typhoid fever 1–4 years previously. We wrote telling them of the possibility of their being chronic *S typhi* carriers and inviting them to come to the Public Health Institute for screening. 3 known male carriers were also included.

Bacteriological evaluation consisted of a stool culture on each of 3 consecutive days and one duodenal-fluid culture. The women were instructed to sample a fresh stool with a sterile swab, inoculate it into Cary-Blair transport medium[13] and to bring the sample to the bacteriology laboratory within 24 h. Samples of duodenal fluid containing bile were obtained by means of a gelatin-encapsulated string device ('EnteroTest', Hedeco) ingested by the subject under supervision.[14] Stools and bile-stained duodenal fluids were inoculated onto MacConkey, Wilson-Blair, and *Salmonella-Shigella* agar, and into selenite F broth. *S typhi* was identified by standard biochemical and serological reactions.[15] A serum sample was obtained from each subject.

We also obtained serum samples from 29 patients of both sexes, aged 18 or over, admitted to the Infectious Diseases Hospital with bacteriologically confirmed typhoid fever and from 59 healthy subjects of both sexes, aged 16–46, who had no bacteriological investigation.

Serum antibodies were measured by the passive haemagglutination assay method of Nolan *et al*[8] with highly purified Vi antigen prepared from *Citrobacter freundii* by a modification of Wong and Feeley's technique[7] (provided by J. B. Robbins, Division of Bacterial Products, National Center for Drugs and Biologics, USA). Serum samples were first allowed to react with unsensitised sheep erythrocytes to absorb anti-sheep-cell antibodies.[16] Glutaraldehyde-treated sheep erythrocytes[17] were sensitised with highly purified Vi antigen (10 μg/ml). Serial two-fold dilutions of serum samples, from 1/20 to 1/2560, were added to equal volumes of sensitised and non-sensitised cells. The agglutination patterns were read after 2 h incubation at room temperature and again after incubation overnight at 4°C. Titres were recorded as the reciprocal final dilution showing a positive haemagglutination result. Known positive and negative control sera were assayed with each test. Student's *t* and chi-squared tests were used for statistical analyses.

RESULTS

Of the 36 chronic carriers (3 known and 33 detected by bacteriological screening), 27 (75%) had Vi titres of ≥160 (see table), whereas only 8% of the 388 non-carrier women ($p<0.001$) and 3% of 59 healthy subjects who had no bacteriological screening ($p<0.001$) had titres ≥160. The

PREVALENCE OF Vi ANTIBODY IN CHRONIC *S TYPHI* CARRIERS, ACUTE TYPHOID FEVER PATIENTS, AND HEALTHY SUBJECTS IN SANTIAGO, CHILE

Group	Sex	Age (yr)	Titre*	Percentage with titre		
				<40	80	≥160
Chronic *S typhi* carriers (n = 36)	92% women	17–59	296	14	11	75
Acute typhoid fever patients (n = 29)	Both sexes	18–30	53	48	14	38
Non-carriers with typhoid fever 1–4 years earlier (n = 38)	All women	25–62	21	85	7	8
Healthy subjects (n = 59)	Both sexes	16–46	16	85	12	3

*Reciprocal geometric mean titre.

frequency of titres ≥160 in patients with acute typhoid fever (38%) was also significantly lower than that in chronic carriers ($p<0.005$). The geometric mean titre in the chronic carriers was significantly ($p<0.001$) higher than that in any of the other groups (table).

The sensitivity and specificity of each Vi antibody titre as a cut-off point in screening for chronic *S typhi* carriage was determined with the 388 culture-negative women as negative controls (fig 1). With a Vi antibody titre of ≥160 taken as positive, the passive haemagglutination assay with highly purified Vi antigen had 75% sensitivity and at least 92% specificity.

The predictive value of each Vi antibody titre as cut-off point in screening for chronic carriers (defined as the percentage of subjects with positive Vi serology who will be confirmed as chronic *S typhi* carriers[18]) was determined in populations with different carrier prevalence rates (fig 2). When we used the 388 culture-negative women with history of confirmed typhoid fever as the negative controls, the specificity of a Vi antibody titre ≥160 was 92%. Thus, in Santiago the predictive value of this titre is 8% in the general adult population, 16% in women 40 years and older, and 37% in women 25 years and older with history of confirmed typhoid fever (fig 2A). However, the 59 healthy Chileans (who were not studied bacteriologically) may be more representative of the general population in Santiago; when they were used as the negative controls, the specificity of a Vi antibody titre ≥160 rose to 97% and the predictive value rose from 8% to 17% in the general adult population and from 16% to 31% in women 40 years and older (fig 2B).

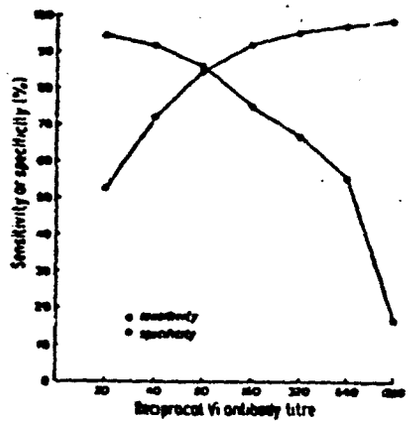

Fig 1—Sensitivity and specificity of various reciprocal Vi antibody titres as cut-off point in screening for chronic *S typhi* carriers.

DISCUSSION

3–5% of patients with typhoid fever become chronic carriers and the carrier state persists throughout life.[14,19] Since man is the only natural host and reservoir of *S typhi*,[19] the detection of chronic carriers is essential for the control of typhoid fever. The use of bacteriological cultures for the detection of chronic *S typhi* carriers is limited by expense, logistical considerations, and the fact that carriers typically have intermittent excretion of *S typhi* so repeated cultures are necessary.[11] Some reports of the non-surgical treatment of chronic *S typhi* carriers have been encouraging,[20,21] so the use of Vi serology to screen for carriers in selected high-risk

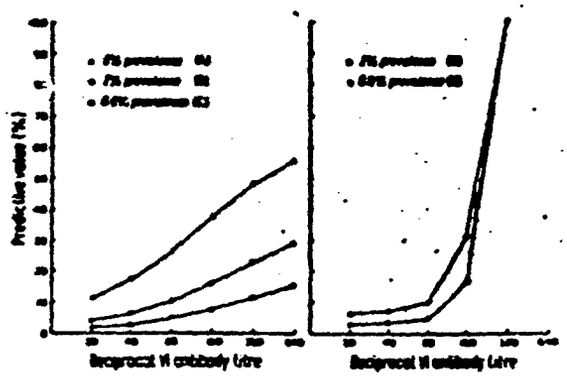

Fig 2—Predictive value of various reciprocal Vi antibody titres as cut-off point in screening for chronic *S typhi* carriers in populations with different carrier prevalence rates.

Left-hand panel = culture-negative women ≥25 years with documented typhoid fever 1–4 yr earlier as negative controls. *Right-hand panel* = healthy adult population, both sexes, as negative controls.

(A) Women ≥25 years with documented typhoid fever 1–4 yr earlier (C. F. Lanata, unpublished). (B) women ≥40 yr;[10] (C) both sexes ≥40 yr.[10]

groups in an endemic area may be justified as part of a programme to control typhoid fever.

The main reason for the conflicting reports of the usefulness of Vi serology in the detection of chronic *S typhi* carriers is the differing purity of the Vi antigen. The original studies with direct bacterial agglutination[1,2] used an *S typhi* strain rich in Vi as antigen. This strain, however, also contained somatic O and flagellar H antigens so the sera had to be preabsorbed with a Vi-negative *S typhi* strain. The isolation of *S typhi* strain Vi 1,[22] which was rich in Vi antigen but almost without O and H antigens, simplified direct bacterial agglutination. However, both the direct bacterial agglutination test and the passive haemagglutination assay with crude[3,6] or partially purified Vi antigen[5,10] have a high false-positive rate in the general population, especially where typhoid fever is endemic, apparently because of cross-reactivity with other antigens in these antigen preparations.[16] The lack of specificity has been the reason for the loss of confidence in Vi serology since the late 1950s.

The development of a method to produce highly purified Vi antigen[7] was important, since it provided an antigen that improved the specificity of the technologically simple passive haemagglutination assay.[8,9,23] A preliminary report[23]

suggested that the passive haemagglutination assay with highly purified Vi antigen had no greater sensitivity or specificity than direct bacterial agglutination and more elaborate techniques, such as fluorescent Vi antibody test,[23,24] counter-immunoelectrophoresis, or solid-phase radioimmunoassay[25] have been advocated. However, these more sophisticated techniques require expensive equipment and their use in less-developed countries where typhoid fever is endemic is limited.

The practical application of the simple passive haemagglutination assay with highly purified Vi antigen in detecting chronic *S typhi* carriers in an endemic area depends not only on the sensitivity and specificity of the titre used as cut-off but also on its predictive value.[10] Since the predictive value of each titre cut-off is greater in populations with higher chronic *S typhi* carrier rates, screening high-risk groups of the population will be justifiable.

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Child Health

USE OF NORMAL IMMUNOGLOBULIN IN AN ECHOVIRUS 11 OUTBREAK IN A SPECIAL-CARE BABY UNIT

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Summary An epidemic of echovirus 11 infections occurred in the Cambridge special-care baby unit during August to October, 1982. There were 21 confirmed infections in babies; 1 died, 1 recovered after resection of a kidney, 5 had meningitis, and 6 had respiratory symptoms. Normal human immunoglobulin which contained antibody to echovirus 11 was administered intramuscularly (250 mg) to give protection. None of the children given immunoglobulin immediately after delivery (205 doses) developed symptoms or ill-effects. Serological studies reinforced earlier evidence for the protective action of antibody, and it is considered that immunoglobulin is a valuable safeguard for exposed newborn infants.

INTRODUCTION

THE first description of echovirus 11 in 1959 was followed by several reports of infections in newborn infants[1-3] but we found only one report of a death in a child under 1 year.[4] In 1977 the potential seriousness of echovirus 11 infection was shown by an outbreak in the Cambridge special-care baby unit, in which 3 of 9 infected infants died.[5] This outbreak heralded a widespread epidemic in England and Wales with 1495 infections recorded in patients of all ages in 1978, nearly double the 895 recorded in the previous 10 years.[6] Infection spread to countries of both hemispheres[7,8] and included cases in newborn infants in several nurseries in the UK[9] (and unpublished observations by E. W. Colley and by D. B. Weldon and J. Nagington) and fatal infections in the USA.[10]

A retrospective study of deaths in children in England and Wales during 1968 to 1978[6] showed that at least 12 in babies who died aged 5–11 days were caused by echovirus 11. All died within 24–48 h of the onset of signs with an overwhelming virus infection characterised by disseminated intravascular coagulation and haemorrhage into organs, especially the renal medulla and adrenal glands. A second group of 12 died aged between 9 weeks and 4 years 10 months with less clearly definable symptoms, mainly respiratory; six of these were recorded as cot deaths.

The techniques that now permit premature infants to be successfully cared for in special-care baby units have created groups of children susceptible to serious epidemic infection from enteroviruses such as echovirus 11. If, as seems likely, the introduction of such infections cannot be prevented, there is a pressing need to protect the infants from the consequences. We now report an echovirus 11 outbreak, of much greater extent than the 1977 outbreak, in which normal human immunoglobulin was used to protect the babies and to enable the ward to carry on with the minimum of disruption.

Development and Evaluation of an Enzyme Linked Immunosorbent Assay for Serum Vi Antibodies for Detection of Chronic *Salmonella typhi* Carriers

Running Title: ELISA Vi Antibodies to Detect *S. typhi* Carriers

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Abstract

An enzyme linked immunosorbent assay (ELISA) measuring serum IgG, IgM, and IgA antibodies to Vi capsular polysaccharide antigen that had been tyraminated to increase its binding efficiency to microtiter plates (Vi-Tyr) was compared to the standard passive hemagglutination assay (PHA) as a screening test for chronic Salmonella typhi carriers. Initially, three populations were evaluated: 22 healthy U.S. adults, 17 young Chilean adults with acute typhoid fever, and 51 Chileans who had bacteriologically confirmed S. typhi chronic carriage. IgG specific Vi-Tyr antibodies were preferentially present in the S. typhi chronic carrier state. 44/51 (81%) chronic carriers, 0/22 (0%) healthy U.S. adults, and 2/17 (12%) Chileans with acute typhoid fever had reciprocal serum IgG Vi-Tyr ELISA antibody titers $\geq$ 200. The IgG Vi-Tyr ELISA was then compared to the PHA as a screening test for chronic carriers in 141 Chilean female foodhandlers. One woman was serologically incriminated as a carrier by both the IgG ELISA and PHA; her coprocultures were positive for S. typhi. One other woman, identified as a carrier by PHA was negative by culture and IgG ELISA. The IgG Vi-Tyr ELISA is as sensitive as the PHA (86% vs 76%) and as specific (95% vs 95%) in screening for chronic carriers.

Asymptomatic excretion of Salmonella typhi in stools for greater than one year following an episode of acute typhoid fever occurs in approximately three percent of adults (9). These asymptomatic chronic biliary carriers represent an important reservoir of S. typhi and have been responsible for outbreaks of acute typhoid fever (13). Detection of carriers, therefore, becomes an important aspect of typhoid fever control. Bacteriological confirmation of the chronic carrier state requires either multiple stool cultures or cultures of bile or bile-stained duodenal fluid. These procedures are not amenable to large scale screening (10,7,6). In addition, because chronic biliary carriers are often intermittent or light fecal S. typhi excretors, multiple bacteriological examinations are usually required to reliably make the diagnosis (10,6,3). For these reasons, serologic screening for the carrier state of Salmonella typhi in areas of typhoid endemicity is preferable.

The passive hemagglutination assay (PHA), using Vi antigen from Citrobacter freundii or S. typhi, has been found to be both sensitive and specific for the screening of the chronic carrier state of S. typhi in endemic and non-endemic areas (8,11). However, this assay requires test sera to be pre-absorbed with sheep erythrocytes, which is inconvenient in screening large populations. Attempts at using an enzyme linked immunosorbent assay (ELISA) for the detection of the carrier state have been hampered by the poor binding of the Vi antigen to microtiter plates. Some researchers, using immune sera as the capture reagent for the Vi antigen, have had some success in detecting specific IgG antibodies (8,11). However, large amounts of a standa / immune sera are needed and may not be readily available.

Highly purified Vi polysaccharide from Citrobacter freundii was tyraminated (Vi-Tyr) in an attempt to enhance binding of the polysaccharide to plastic microtiter plates (12). This Vi-Tyr was then used in the development

of an ELISA which was compared to the passive hemagglutination assay (PHA) as a screening test for typhoid carriers in a typhoid endemic area. The ELISA was adapted to assess the relative occurrence of IgG, IgM, and IgA Vi specific antibodies in the carrier state.

Materials and Methods

Subjects. To standardize the Vi-Tyr ELISA, 3 groups of subjects were examined: 22 healthy young adults from the United States, 17 young Chilean adults admitted to the Infectious Diseases Hospital in Santiago with bacteriologically confirmed acute typhoid fever, and 51 asymptomatic S. typhi carriers from Chile. These chronic carriers had bacteriologically confirmed typhoid fever 1-4 years previously and, at the time of the study, had S. typhi isolated bacteriologically.

Once a serum dilution for screening carriers was determined using these known populations, 141 Chilean female foodhandlers, age 25-65 years, were screened blindly for S. typhi chronic carriage using the Vi-Tyr ELISA and PHA. S. typhi carriage in this group was confirmed by coprocultures.

Specimens. One serum sample was obtained from each subject in all groups except for the subjects with acute typhoid fever who had sera obtained upon hospital admission and 21 days later. Those subjects with acute typhoid fever and those with chronic S. typhi carriage had bacteriologic evaluation consisting of three stool cultures obtained on consecutive days and one duodenal fluid culture obtained by a gelatin-encapsulated string device (1). Two coprocultures were obtained on successive days from the healthy female foodhandlers to confirm S. typhi carriage. All samples were inoculated onto

MacConkey, Wilson-Blair, and Salmonella-Shigella agar, and into Selenite broth. S. typhi was recovered and identified by standard biochemical and serological reactions (4).

Tyramination of the Vi antigen. The tyramination of the Vi polysaccharide has been previously described (12). Briefly, tyramine (30 mg/ml) was added to 10 mg Vi in the presence of carbodiimide and incubated at pH 4.9 -5.1 for 3 hours. The resultant reaction mixture was dialyzed and purified by gel exclusion through a G-100 Sephadex column (Pharmacia, Piscataway, NJ).

Standardization of the Vi-Tyr ELISA. Sera from 16 known chronic typhoid carriers and from 6 healthy U.S. volunteers were used as the positive and negative reference sera, respectively, to establish a standard curve for each isotype-specific Vi-Tyr ELISA. These samples were assayed twelve different times at two-fold dilutions starting at 1:25 and ending at 1:3200 by the following method:

The wells of Immulon I (Dynatech, Alexandria, Va) plates were incubated at 4°C overnight with 0.1 ml aliquots of Vi-Tyr antigen in phosphate buffered saline (PBS), pH 7.3. The wells were washed 5 times with PBS containing 0.05% Tween 20 (PBS-Tween) and then incubated at 37°C for 1 hour with 0.1 ml of human serum diluted in PBS-Tween containing 1% non-immune goat serum and 1% fetal bovine serum. The wells were then washed 5 times with PBS-Tween and incubated for 1 hour at 37°C with heavy chain specific antibody to human immunoglobulin G, M, and A conjugated to alkaline phosphatase (Kirkegaard and Perry, Gaithersberg, Md.) diluted in PBS-Tween. After washing, the wells were incubated at room temperature with 0.1 ml of p-nitrophenyl phosphate (1mg/ml) in 10% diethanolamine buffer (pH 9.8). Absorbance was monitored at 405nm.

Saturation kinetics using several high PHA titered serum samples were determined using Vi-Tyr coating concentrations of 0.5, 1.0, and 2.0 ug/ml. The specificity of the goat antibody conjugates were examined with purified IgG, IgM serum fractions and milk IgA obtained by filtration through a DEAE Biogel-A (Pharmacia) column.

The PHA was performed for each subject in each group by methods previously described (6). A titer of $\geq$ 160 was considered to be indicative of the S. typhi carrier state.

Results

Assay Standardization. The absorbance (A) of the pooled positive serum was determined as a function of the tyraminated Vi antigen coating concentration. Saturation kinetics were observed and a coating concentration of 1 ug/ml was chosen. The pooled IgG fraction contained 1450 mg/dl IgG and less than 1 mg/dl IgM. The pooled IgM fraction contained 140 mg/dl IgM and less than 1 mg/dl IgG. The IgA sample contained 35.5 mg/dl IgA with less than 1 mg/ml IgG and IgM. The conjugates were shown to be isotype specific.

A standard curve for IgG specific Vi antibody using positive sera was linear for absorbance (A) values ranging from 1.2 to 0.1 using serum dilutions ranging from 1:50 to 1:800 (figure 1). The negative serum pool gave optical densities (O.D.) below 0.1 for dilutions as low as 1:25. A dilution of 1:50 of the negative serum pool produced a mean O.D. of 0.04 with a standard deviation (S.D.) of 0.01. The cut-off absorbance value signifying significant IgG specific Vi antibody was set at an O.D. reading of 0.2 since this value is on the linear portion of the curve and well above background.

The IgM standard curve for Vi antibody was linear for A values ranging

from 1.4 to 0.15 using serum dilutions ranging from 1:25 to 1:400 (figure not shown). Because of high background values in the negative serum pool for dilutions less than 1:1000, starting dilutions of 1:100 were used in all samples. A cut off of 0.3 was used to determine a positive antibody titer since the absorbance of the negative serum pool, at a 1:100 dilution was 0.87 ± 0.05. Similarly, an IgA standard curve was determined as being linear for A values ranging from 0.9 to 0.14 using serum dilutions ranging from 1:50 to 1:400 (figure not shown).. An absorbance of 0.15 was conservatively chosen as the cut-off for a positive antibody titer since the pooled negative sera at a 1:50 dilution gave an O.D. of 0.02 ± 0.01.

Evaluation of the Vi-Tyr ELISA to detect S. typhi carriers. Table 1 shows the results of the IgG Vi-Tyr specific antibody titers in individuals with acute typhoid fever and S. typhi carriage, and a healthy population. Of the 51 chronic carriers tested, 444 (86%) had an IgG Vi-Tyr ELISA titer greater than or equal to 1:200. In contrast, only 12% of the acute typhoid patients and none of the healthy U.S. volunteers had similar titers ($p < 0.00000001$). An IgM specific Vi antibody titer >100 was detected in 19 (37%) of chronic carriers and in 3 (18%) of patients with acute typhoid fever (Table 2). The IgM Vi-Tyr ELISA was unable to discriminate acute typhoid fever patients from chronic carriers ($p = 0.2$; and did not increase the detection sensitivity of carriers of the IgG Vi-Tyr ELISA. Although Vi-Tyr specific IgA antibodies were present in 37 (72%) of the chronic carriers, they also were detected in patients who had acute typhoid fever (Table 3) ($p = 0.2$). ELISA Vi-Tyr antibodies of all three immunoglobulin classes were seen with equal but low frequency in the admission and follow-up serum samples obtained from patients with acute typhoid fever.

To assess the applicability of the IgG Vi-Tyr ELISA in a typhoid endemic area, 141 Chilean female food handlers were screened by the ELISA, PHA, and two coprocultures. Of these 141 women tested, one had an IgG ELISA titer $\geq$ 200 and two women, one of whom also had the positive ELISA titer, had a PHA titer $\geq$ 160 (Table 1). Of these two women serologically identified as possible carriers, only the one woman who was positive by the Vi-Tyr IgG ELISA was confirmed to be a carrier by culture.

The sensitivity of the IgG Vi ELISA titer of $\geq$ 200 in screening for chronic S. typhi carriage as determined by analyzing the results obtained with the 51 known chronic carriers is 86% compared to 76% with the PHA using a titer of $\geq$ 160. The specificity of the IgG specific Vi-Tyr ELISA in screening for chronic carriers using healthy U.S. volunteers and acute typhoid fever patients is 95%, which is equal to that obtained by using the PHA.

Discussion

Since man is the only reservoir of S. typhi, the detection of carriers is necessary for control of typhoid fever. In areas of typhoid endemicity, screening for chronic typhoid carriers by serological means is of practical importance since bacteriologic screening is expensive and logistically difficult to perform. The tyramine derivative of Vi provides sufficient binding of the antigen for detection of Vi specific antibodies by ELISA. In terms of rapidity and ease of performance, we find the IgG specific Vi-Tyr ELISA to be superior to our previously reported PHA for the detection of S. typhi carriers (1,12).

Analysis of the different classes of antibody involved in the chronic carrier state has shown that Vi antibody of the IgG class is present most

frequently. IgM and IgA Vi antibodies, although seen in chronic carriers, cannot be used to differentiate persons with acute or chronic S. typhi infection. It is probable that the IgG antibody response to Vi present in carriers reflects prolonged immunologic stimulation. It is interesting that a serum IgA Vi response is present in both the acute and chronic forms of S. typhi infection. This seems reasonable since S. typhi participates in an enterohepatic circuit in the pathogenesis of acute typhoid fever and also is a primary occupant of the biliary system in chronic infection. Further work on subclass specificities in both the IgG and IgA responses to Vi antigen in the acute and chronic forms of S. typhi infections may help elucidate other possible immunologic differences in these two disease states.

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Figure 1. IgG specific antibody response to Vi-Tyr antigen in pooled sera from 16 asymptomatic Salmonella typhi carriers as measured by ELISA. Antibody results are represented as the mean (dots) and two standard deviations (bars) compiled from 12 separate runs.

TABLE 1

lence of IgG Specific Vi Antibody

Group Descri	VI[a]	ELISA titer[b]			PHA titer[c]	
(No. subjec		<50	50-100	>200	<40	80
U.S. volunteer (22)	5	21	1	0	21	1
Acute typhoid patients (17)	1	15	0	2	14	1
Chronic carrie (51)		6	1	44	7	5
Food handlers (141)		140	0	1[e]	139	0

a = recipr cal n Vi titer

b = number of a given reciprocal Vi titer

c = number of a given reciprocal PHA titer

d = admiss up titer

e = the or ed as being a chronic carrier bacteriological

NA = not a

TABLE 2

Prevalence of IgM Specific Vi Antibody

Group Description (no.)	GMT[a]	ELISA titer[b]	
		<100	>100
U.S. volunteers (22)	43	21	1
Acute typhoid patients (17)	34/40[c]	14	3
Chronic carriers (51)	64	32	19

a = reciprocal geometric mean Vi titer

b = No. subjects with a given reciprocaltiter

c = admission titer/ follow-up titer

TABLE 3

Prevalence of IgA Specific Vi Antibody

Group Description (no.)	GMT[a]	ELISA Titer[b] <50	ELISA Titer[b] >50
U.S. volunteers (22)	18	20	2
Acute typhoid patients (17)	50/51[c]	6	11
Chronic carriers (51)	52	14	37

a = reciprocal geometric mean titer

b = number of subjects with a given reciprocal titer

c = admission titer/ follow-up titer

NON-SURGICAL TREATMENT OF CHRONIC *SALMONELLA TYPHI* CARRIERS WITH AMOXICILLIN AND PROBENICID

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Running Head: Antimicrobial Therapy of Chronic Typhoid Carriers

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SUMMARY

An oral regimen of amoxicillin 2.0 gm combined with probenecid 0.5 gm three times a day for 28 consecutive days was evaluated in the treatment of chronic Salmonella typhi carriers in Santiago, Chile. Mild (mostly gastrointestinal) but tolerable side effects commonly occurred early in the therapy. Fifteen of 26 (58%) treated carriers (including three with gallstones) remained cured after 12 months of bacteriological follow-up; all failures became evident within the first four months after completing therapy. The serum amoxicillin blood level at four hours post-dose was significantly higher (19.50 $\pm$ 2.90 mcg/ml) in the cured carriers compared with the carriers who failed (14.69 $\pm$ 2.77 mcg/ml) ($p<0.01$). This regimen provides a reasonable alternative to cholecystectomy in selected carriers. However, the cure rate of 58% is arguably too low to justify systematic use in typhoid fever control programs.

INTRODUCTION

Approximately 3% of patients with typhoid fever become chronic gallbladder carriers and thereby serve as a reservoir for the transmission of Salmonella typhi (Ames & Robins 1943, Anderson et al., 1936). The combination of cholecystectomy and antibiotics is largely successful in eradicating the carrier-state (Munnich & Bakemi 1979, Perkins et al., 1966, Whitby 1964). However, because this regimen is invasive and expensive, it is not a practical public health tool. Based on these drawbacks, investigators have studied the effectiveness of antibiotics alone in eradicating the carrier state, using drugs to which S. typhi are susceptible in vivo. Overall, medical therapy alone has been disappointing and success has generally been correlated with the presence or absence of gallstones (Bullock 1963, Dinbar et al., 1969, Kaye et al., 1967, Munnich et al., 1974, Nolan & White 1978, Scioli et al., 1972, Simon & Miller 1966, Tynes & Utz 1962). Nevertheless, two reports have generated optimism. Scioli et al (1972) successfully treated all of 19 chronic S. typhi carriers with a 15 day course of intravenous ampicillin, suggesting that high cure rates are achievable if sufficiently high serum and biliary levels of a bactericidal antibiotic can be maintained. Because of the impracticality of parenteral therapy, regimens are being sought to achieve this with an oral antibiotic. Amoxicillin, a congener of ampicillin which gives two to three-fold greater blood levels after oral dosing and is concentrated in bile, was considered an attractive drug to be evaluated in treatment of the S. typhi carrier state (Kosmidis et al., 1972, Waki et al., 1977). Nolan & White (1978) treated 15 carriers with amoxicillin, 6.0 gm/day. Of 10 carriers who completed this 28 day regimen, nine were cured, including three with gall bladder disease. The remaining five carriers had their amoxicillin dose reduced to 3.0 gm/day because of gastrointestinal

intolerance; of these, only two were cured.

Based on this background, we conducted a clinical trial in Santiago, Chile, where typhoid fever is highly endemic and the prevalence of chronic carriers and cholelithiasis is high (Levine et al., 1982), to evaluate an oral antibiotic regimen that might cure S. typhi carriers without surgery, regardless of the presence of gallstones. In this trial, amoxicillin was combined with probenecid to increase and prolong the amoxicillin blood levels.

PATIENTS AND METHODS

Subjects

Twenty-six bacteriologically-confirmed chronic S. typhi carriers who were free of any debilitating disease, did not have history of penicillin allergy, gastrointestinal or renal disease and were not pregnant or lactating women were enrolled into the study (Lanata et al., 1983). These included 22 persons who were known carriers for at least 12 months, two for nine months, and two who were carriers for six months. Written informed consent was obtained.

Bacteriology

All participating carriers had a baseline medical evaluation. Prior to treatment a stool culture on each of three consecutive days and one duodenal-fluid culture were obtained. The carriers were instructed to inoculate a sample of fresh stool with a sterile swab into Cary-Blair transport medium (Finegold & Martin 1982) and to bring the sample into the bacteriology laboratory of the Institute of Public Health within 24 hours. Samples of duodenal fluid containing bile were obtained by means of a gelatin-encapsulated string device ("Entero-Test", H.D.C. Corporation, Mountain View, Ca.) ingested by the subject under supervision (Gilman et al., 1979, Avendano et al., 1986). Stools and bile-stained duodenal fluids were inoculated onto MacConkey, Wilson-Blair, and Salmonella-Shigella agar

directly, as well as after 18 hours enrichment in selenite F broth. S. typhi was identified by standard techniques (Edwards & Ewing 1972).

After treatment, three consecutive stool cultures and one duodenal-fluid culture were obtained during the first week post-treatment and at the 1st, 3rd, 6th, 9th and 12th month post-treatment, whenever possible. Carriers with all cultures free of S. typhi for 12 or more months after completion of therapy were considered cured.

Treatment Regimen

The oral treatment regimen consisted of amoxicillin trihydrate, 2.0 gm and probenecid 0.5 gm taken three times each day for 28 days by the patient at home. To detect side effects and evaluate compliance, each carrier was contacted at least twice a week by telephone or by home visits. A diary was provided for the carriers to record the precise times when they took their medication and the appearance of any side effects. The medication was provided in single-dose vials, to assure ingestion of the correct number of capsules. A spot urine sample was obtained during the home visits to be subsequently tested for amoxicillin levels as an indicator of compliance. Finally, during the second and last week of treatment, a serum sample was obtained before the morning dose, and two, three and four hours thereafter for amoxicillin blood levels.

Amoxicillin Levels

Sera and urine samples were frozen at -70°C and transported on dry ice to Baltimore. Because of the loss of one box of specimens during transport, samples were available from only 20 carriers. The serum and urine amoxicillin levels were determined in quadruplicate on a gel-diffusion system using five known standard dilutions in each plate (Bennett et al., 1966).

RESULTS

Twenty-six carriers (92% women) infected with amoxicillin-sensitive S. typhi were enrolled; their mean age was 34.6 years (Table 1). The gallbladder status was known in 15 carriers (13 consented to have cholecystograms): none had a normal gallbladder; eight had gallstones; five had a non-functioning gallbladder; two others were hepatic biliary tree carriers who had had cholecystectomies four and 12 months earlier.

Side Effects

Most of the carriers (88%) had mild and transient adverse reactions during the first week of therapy that disappeared without interrupting the antibiotic treatment. In total, 13 (50%) carriers complained of mild epigastric pain, lasting a mean of 2 days; 11 (42%) experienced nausea, enduring a mean of 2.5 days; seven (27%) had diarrhoea, persisting for a mean of 5.4 days; six (23%) had a diffuse pruritic rash, lasting a mean of 7.7 days; and six (23%) had other symptoms. Two carriers developed intense epigastric pain that led to interruption of treatment. When the same treatment schedule was re-initiated five to seven days afterwards combined with antiacids, mild epigastric discomfort recurred in one women which lasted four days.

The unscheduled home visits demonstrated a high degree of compliance. On each visit each carrier had the correct number of unused individual doses. Of the urine samples available for testing from 20 carriers, multiple samples tested from 19 carriers had a high urinary level of amoxicillin; the remaining carrier had one of five urines negative for the drug.

Bacteriological Response to Therapy

After a period of 12 or more months of follow-up, 15 of the 26 carriers (58%) persisted with negative cultures for S. typhi and were considered cured

(Table 1). Notably, this group included the two intrahepatic biliary S. typhi carriers (McFaddzean 1966). Three (38%) of the eight carriers known to have gallstones and one (20%) of the five who had a non-functioning gallbladder were cured. When the probability of cure was observed by month post-treatment, it became stable after the 4th month (Figure 1). No failures occurred after that period.

Various parameters were analyzed to compare the carriers who failed with those who were cured. No differences were found with respect to age or duration of carriage prior to therapy (Table 1). Furthermore, the proportion of carriers who developed side effects was similar between the two groups.

One objective difference between the groups was found in the serum amoxicillin levels of the 20 carriers whose specimens were available for testing. There were no differences in the serum amoxicillin levels between the 12 cured and eight failed carriers at baseline (overnight level), or two and three hours after the morning dose (Figure 2). However, the mean serum amoxicillin level at four hours after the morning dose in the 12 tested carriers who were cured (19.50 $\pm$ 2.9 mcg/ml) was significantly higher than the mean level among the eight tested carriers who failed (14.69 $\pm$ 2.77) ($p<0.01$, Student's t test) (Figure 2).

DISCUSSION

After 28 days of 6.0 gm of amoxicillin combined with 1.5 gm of probenecid daily, we successfully eradicated S. typhi in 15 (58%) of 26 chronic carriers who were highly compliant in taking their medication. Our results are somewhat in contrast with those of Nolan & White (1978) who obtained a 90% cure rate among 10 carriers treated for the same period of time with the same amoxicillin dosage but without probenecid. Because of the small numbers involved, the difference in cure rates (nine of 10 versus 15 of 26) may very

well be due to chance ($p=0.11$, two-tailed Fisher's Exact test). However, the most likely explanation of the difference in results is the high prevalence of gall bladder disease among our carriers. All 13 carriers in our series who had oral cholecystograms exhibited cholelithiasis or a non-functioning gall bladder, in contrast with only 3 of 10 carriers in the report by Nolan & White (1978) ($p<0.005$) who were treated with the same amoxicillin regimen but without probenecid. Others have noted the relationship between gallstones and failure rate in chronic carriers treated with antibiotics (Bullock 1963, Dinbar et al 1969, Tynes & Utz 1962).

Nolan & White (1978) reported a significantly lower serum level of amoxicillin six hours after the last treatment dose in the carriers who failed treatment. Our results corroborate the importance of high and prolonged antibiotic levels in achievement of cures: a significantly lower mean serum amoxicillin level was observed at the 4th hour post-dose among the carriers who failed, in comparison with the carriers who were cured (Figure 2).

We conclude that 2.0 gm of amoxicillin combined with 0.5 gm of probenecid given three times a day for 28 days has a definite but limited role in the ambulatory, non-surgical treatment of chronic S. typhi carriers. In providing a >50% cure rate, this therapeutic regimen offers to the sporadic chronic carrier a moderate chance to eradicate the carrier state without resort to surgery. This is particularly relevant for carriers who, because of other conditions, are deemed unacceptable surgical risks. In contrast, the cure rate of 58% with this regimen is probably insufficiently high to justify its use as a major component of typhoid fever control programs. Now that a practical screening test is available to identify chronic typhoid carriers in endemic areas (Lanata et al., 1983), an important intervention in control programs must be the identification and treatment of carriers in

epidemiologically relevant groups such as foodhandlers. Therefore, the search must continue to identify a practical, non-surgical, ambulatory treatment for chronic S. typhi carriers that achieves ≥80% cure rate without causing notable adverse reactions.

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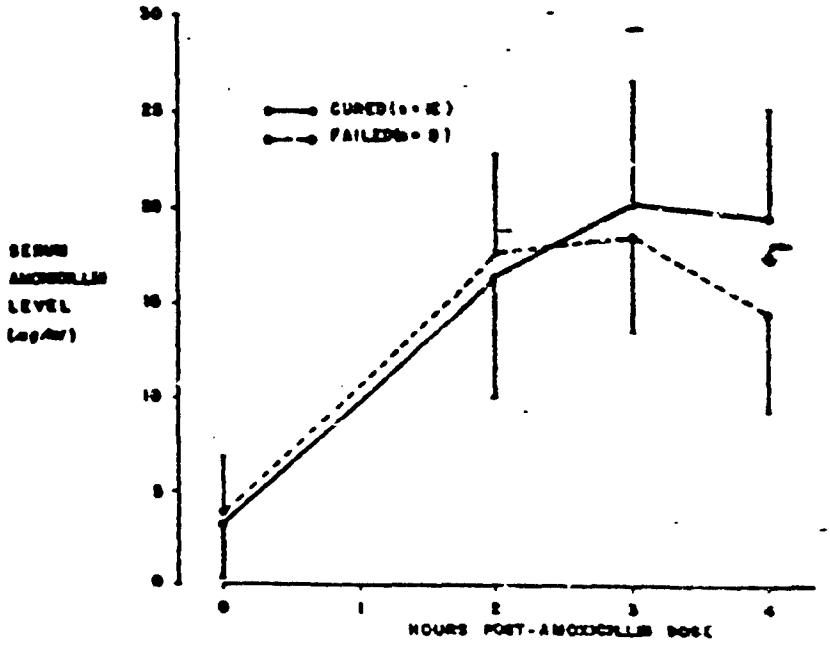
LEGENDS

Figure 1 - The probability of cure (±95% confidence interval) by month post-treatment among 26 chronic Salmonella typhi carriers in Santiago, Chile treated with amoxicillin and probenecid. Data shown as a Kaplan-Meier curve.

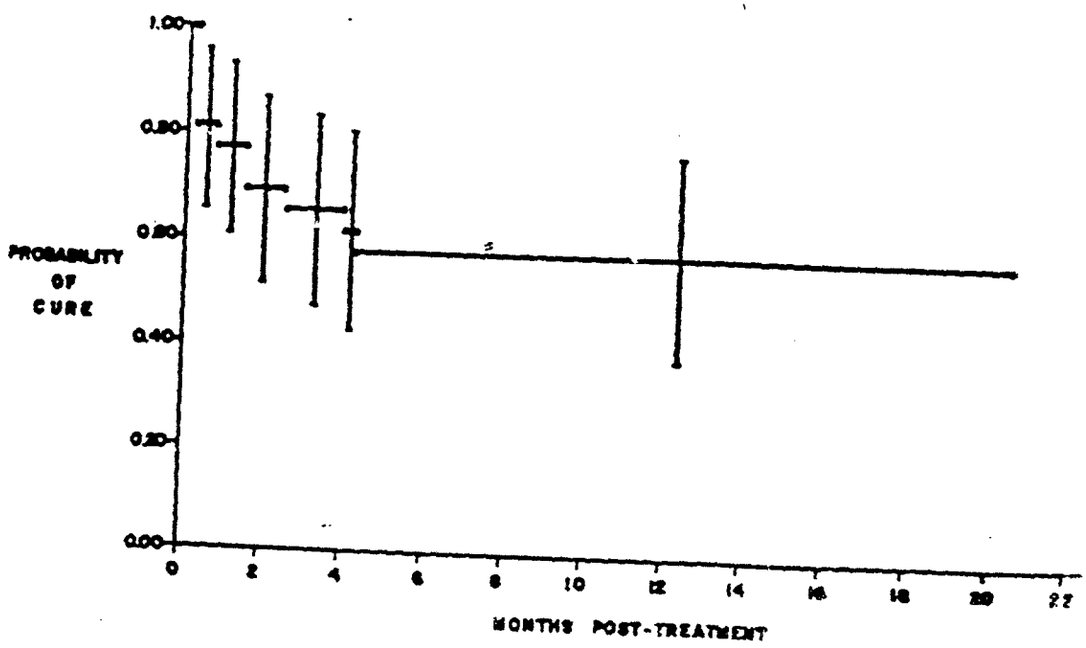
Figure 2 - Mean serum amoxicillin levels (±95% confidence interval) in 20 chronic Salmonella typhi carriers after one 2.0 gm oral dose of probenecid. Data arranged according to carriers who were cured (n=12) or who failed (n=8) on this antibiotic regimen.

SERUM AMOXICILLIN LEVEL (± 95% CONFIDENCE INTERVAL) OF 20 CHRONIC S. TYPHI CARRIERS AFTER ONE ORAL DOSE OF 2 Gm OF AMOXICILLIN AND 0.5 Gm OF PROBENECID ACCORDING TO TREATMENT OUTCOME SANTIAGO, CHILE 1981-1983

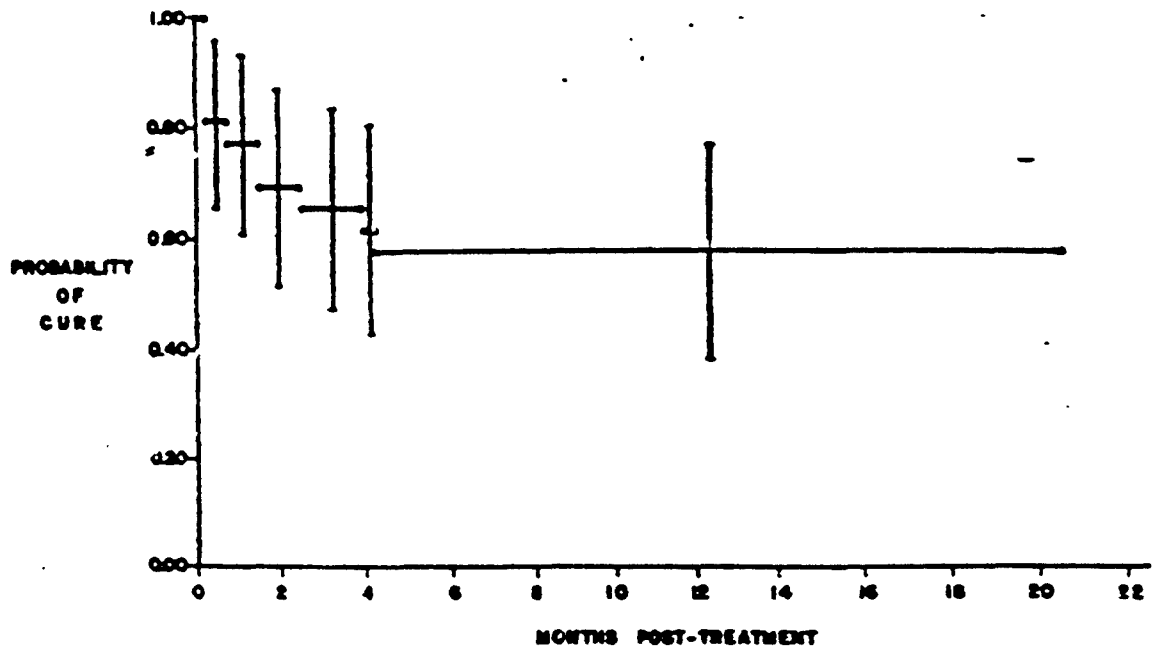


* MEANS DIFFERENCE AT 4 HOURS SIGNIFICANT AT P<0.05, STUDENT'S T TEST

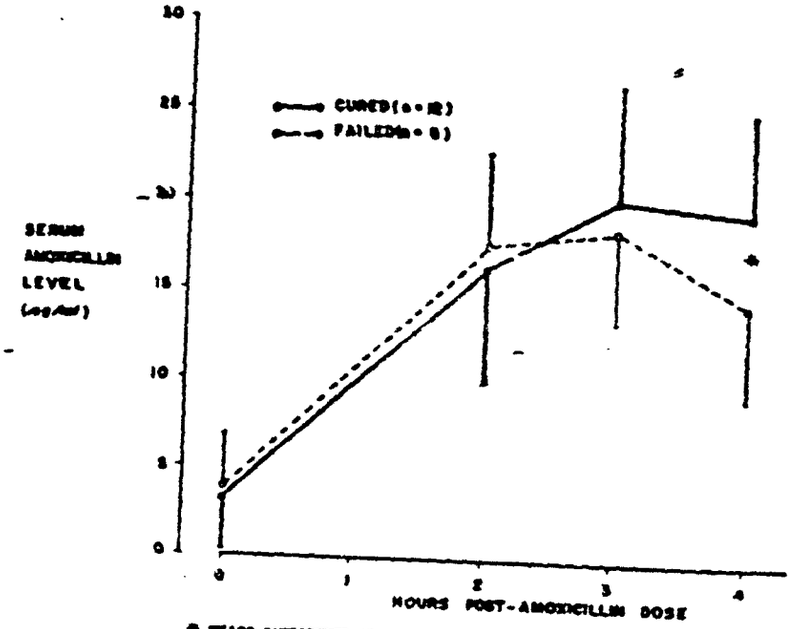
PROBABILITY OF CURE (± 95% CONFIDENCE INTERVAL) BY MONTH POST-TREATMENT OF 3
CHRONIC SALMONELLA TYPHI CARRIERS TREATED WITH
AMOXICILLIN AND PROBENECID - KAPLAN - MEIER CURVE
SANTIAGO, CHILE 1981 - 1983



PROBABILITY OF CURE (± 95% CONFIDENCE INTERVAL) BY MONTH POST-TREATMENT OF 26 CHRONIC SALMONELLA TYPHI CARRIERS TREATED WITH AMOXICILLIN AND PROBENECID - KAPLAN - MEIER CURVE SANTIAGO, CHILE 1981-1983



SERUM AMOXICILLIN LEVEL (± 95% CONFIDENCE INTERVAL) OF 20 CHRONIC *S TYPHI* CARRIERS AFTER ONE ORAL DOSE OF 2 gm OF AMOXICILLIN AND 0.5 gm OF PROBENECID ACCORDING TO TREATMENT OUTCOME SANTIAGO, CHILE 1981-1983



* MEANS DIFFERENCE AT 4 HOURS SIGNIFICANT AT P < 0.01, STUDENT'S T TEST

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The Use of Moore Swabs for Isolation of *Salmonella typhi* from Irrigation Water in Santiago, Chile

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In Chile, a country with an exceedingly high incidence of typhoid, untreated sewage is applied directly to fields where salad vegetables are cultivated. Water used for irrigation was examined for the presence of *Salmonella typhi*, by making use of the sewer-swab technique. *S typhi* was isolated in 8 (11%) of 76 irrigation samples examined from nonindustrial, polluted water. This supports the hypothesis that crops grown with water contaminated with feces are important vehicles in the transmission of *S typhi* in this endemic area. Since sewage treatment plants will not be available in Santiago in the near future, emphasis is being placed on devising alternative methods of irrigation and on growing vegetables that are cooked before being eaten.

Typhoid fever is a major health problem in Santiago, Chile where the annual incidence has exceeded 150 cases per 100,000 population since 1977, with most cases occurring in summer [1]. This is unexpected because Chile has demographic features and health statistics consistent with a technologically advanced society: 94% of homes have bacteriologically monitored, chlorinated water, and 75% have flush toilets [2]. However, human waste is discharged without treatment into the local river, water from which is used to irrigate farmland during the dry summer months. Crops such as lettuce, cabbage, and celery grown with sewage-contaminated water may play an important role as vehicles of *S typhi* when they are consumed raw by residents of Santiago.

Multiple bacteriologic examinations of irrigation water in Santiago have demonstrated high fecal coliform counts and many other *Salmonella*, but no *S typhi* [3, 4]. Although the microbiological methods used in previous attempts were appropriate, optimal sampling and concentrating techniques were not used. The Moore swab, described in England in 1948 [5], is a concentrating method that has been used successfully to locate chronic *S typhi* carriers by isolating the organism from sewage effluents [6, 7]. Its main use has been in the investigation of urban typhoid fever outbreaks [8, 9], and its efficacy and reliability in endemic areas are unknown. We used a modified Moore swab to isolate *S typhi* from environmental sources in Santiago.

Materials and Methods

Microbiological examination of rivers and irrigation canals of Santiago, Chile was carried out from January to March, 1983. The two major waterways in Santiago that carry wastewater are the Mapocho River in the north and Zanjon de la Aguada canal in the south (figure 1). Untreated sewage flows directly into these waters, which are used for irrigation in the agricultural districts of Maipu and Pudahuel (on the perimeter of the city). The Zanjon de la Aguada, which is heavily contaminated with industrial waste from the central section of the city, receives untreated sewage and becomes polluted with feces as it flows westward. In the final few kilometers before it reaches the

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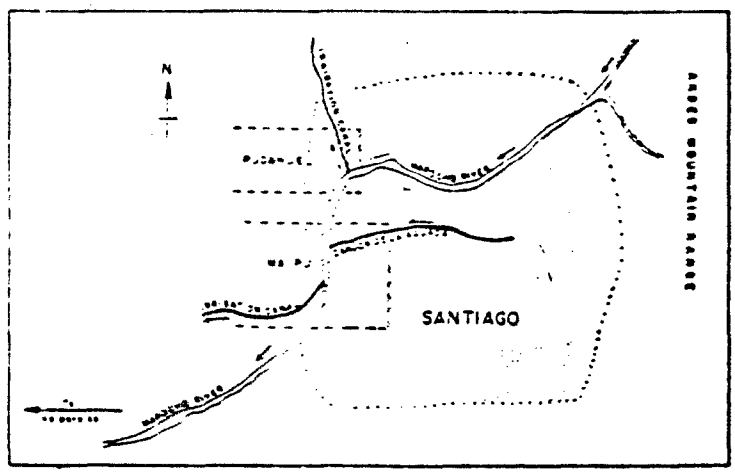

Figure 1. Irrigation system of Santiago, Chile and surrounding farmlands.

agricultural areas, no further sewage is discharged, in an attempt to allow a degree of self-purification of the wastewater. Moore swabs were prepared by wrapping cotton gauze, 15 cm wide by 120 cm long, around wire. The swabs were tied to nylon cord, and suspended in the flowing water of the Zanjon de la Aguada, Mapocho River, and their tributaries. Swabs were also placed directly into irrigation canals of selected farms.

After 48–72 hr, the swabs were removed and immediately placed into 500 ml of Selenite-F broth. The selenite broth was incubated at 41 C and subcultured at 24 hr and 48 hr on to salmonella-shigella, bismuth-sulfite, and deoxycholate-citrate agars. (All broth and media were from BBL Microbiology Systems, Cockeysville, Md.) Suspicious colonies were placed on triple-sugar-iron agar slants and confirmed as *S typhi* by standard methods [10]. *S typhi* isolates were sent to the Central Public Health Laboratory, Colindale, U. K. for phage typing.

Results

We placed 56 swabs into the Mapocho River and 77 swabs into the Zanjon de la Aguada, but recovered only 93. Most lost swabs were due to interference by passersby, who removed or cut the swab. After the first month, by camouflaging the swabs, we were able to decrease losses. Of the 48 swabs recovered from the Zanjon de la Aguada, 17 came from central city industrial areas where there is heavy chemical pollution, and 31 were from agricultural areas where there is a predominance of fecal contamination. None of the 17 swabs from industrial areas and 4 (8.3%) of 45 swabs from the Mapocho River contained *S typhi*. Of the 76 swabs placed in agricultural areas, 8 (11%) were culture positive. Five of the eight isolates were phage-type E1 and 46, the two most common disease-causing types in Chile, one strain was untypeable, and the other two were N and M1.

Discussion

Using Moore swabs, we were able to isolate *S typhi* from irrigation water in Santiago, Chile. To our knowledge, this is the first time Moore swabs have been used for this purpose. The sensitivity of the Moore swab is thought to have an inverse relationship to the flow volume of the waterway sampled [7]. Thus, our isolation rate of 11% from these large waterways is probably an underestimate. *S typhi* is fastidious, easily inhibited by coliforms, and usually present in relatively small numbers in environmental samples [8]. The Moore swab, by acting as a filter, improves the chance of isolating rare *S typhi* among millions of coliforms and has been useful in isolating *S typhi* from sewers during outbreaks of infection in industrialized nations. We have now shown that it is both a practical and reliable epidemiological tool with which to isolate *S typhi* from irrigation water in endemic areas.

Finding *S typhi* with the same phage types as disease-causing isolates in irrigation water supports the hypothesis, based on epidemiological observations, that contaminated vegetables in Santiago serve as important vehicles of transmis-

sion (M. Levine, unpublished data). These observations are as follows: (*1*) typhoid fever peaks during summer when rainfall is lowest and irrigation is used most heavily; (*2*) in the agricultural lake-region of southern Chile where rain water is available all year, there is little irrigation and typhoid fever has a low incidence; (*3*) persons from all socioeconomic groups in Santiago have a high incidence of typhoid fever, a finding suggesting vehicles for infection that are consumed in all areas of the city; and (*4*) bacteriologically monitored, chlorinated water is available in 94% of all households and is thus an unlikely vehicle for *S typhi*.

Enteric diseases can be transmitted by vegetables contaminated by polluted water [11], but a cause and effect relationship is difficult to prove. A study of kibbutzim in Israel showed that communities that practiced wastewater irrigation had a two-to-four times higher incidence of enteric infections [12]. Although our study does not prove that *S typhi* cultured from irrigation water is directly responsible for typhoid fever, its presence implies that an association likely exists between *S typhi*-contaminated vegetables and infection. Recently the government of Chile has intervened to change the farming patterns and usage of contaminated irrigation water. In Maipu and Pudahuel, water from the Mapocho River and the Zanjon will no longer be used to irrigate salad vegetables that can become contaminated and serve as vehicles of transmission of *S typhi*.

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Sensitivity of Moore Sewer Swabs for Isolating *Salmonella typhi*

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Moore swabs (sewer swabs) have been used successfully to culture pathogenic organisms from wastewater. Sensitivity seems to depend on the size of the waterway sampled as well as the number of organisms present. In Santiago, Chile, we placed 24 swabs into the sewers draining the homes of 10 known chronic carriers of typhoid. Swabs were positive for *Salmonella typhi* in 5 of the 10 households (50%) and 6 of the 24 swabs placed (25%).

In 1948, Moore used large gauze pads (sewer swabs) to isolate *Salmonella paratyphi* B from sewage outflow of a coastal English village (6). Two years later, using the swab technique, he was able to isolate *Salmonella typhi* and locate the home of a chronic typhoid carrier (7). The swabs were collected 48 h after placement and cultured in Selenite enrichment broth, with subculturing on Wilson and Blair solid medium (WB). By placing swabs in various sizes of sewers, Moore was able to trace back the source of contamination. He suggested that this technique was most successful when sewer swabs were placed in medium-sized sewers since the sensitivity seemed to be inversely related to the diameter of the sewer sampled (8).

The Moore swab, placed into flowing sewer water, apparently acts as a filter to trap and concentrate pathogenic organisms. The swab shows a more accurate microbiologic composition of the wastewater than water samples since the swab reflects the sum of organisms which have passed through it over time. The Moore swab has been used successfully to isolate viruses, mycobacteria, salmonellae, and vibrios from sewage (1, 4) and has proven useful for investigating the epidemiology of typhoid fever, including typhoid epidemics in industrialized nations (10) and studies of endemic typhoid in Chile (9).

The observation of Moore regarding the relationship of the effectiveness of his swab to sewer size was reconfirmed in the 1964 typhoid outbreak in Aberdeen, Scotland, in which Callahan and Brodie (2) found the sewer swab to be an insensitive tool for random sampling of large sewers. More recently, Barrett et al. (1) found the Moore swab to be both a practical and a sensitive tool for the isolation of *Vibrio cholerae* 01 from relatively small sewers. In a previous study, Sears et al. reported that Moore swabs can be used successfully to isolate *S. typhi* from polluted irrigation water in areas with endemic typhoid fever (9). To evaluate further the sensitivity and reliability of the Moore swab, we placed swabs in the small sewers draining the homes of known, chronic carriers of *S. typhi*.

As part of the projects designed to control typhoid fever in Chile, studies have been performed to locate chronic carriers of *S. typhi*. Through one of these previous studies, which evaluated the efficacy of amoxicillin therapy for treatment of the carrier state, a registry of chronic carriers was compiled. Ten carriers, who were unable to participate in this drug trial, were identified, and permission was obtained to place Moore swabs in the outflow of the sewers draining their homes. The houses of these carriers had flush toilets connected to terra cotta or open pipe drainage. In the front yard of each home was an access panel to the sewer. Most of the houses shared a common sewer with at least one and often two other houses. Swabs were placed directly in the sewers of the homes of the 10 carriers and left for 48 or 72 h. Each sewer was sampled at least two (and usually three) separate times, and an effort was made to assure that the carriers remained home during the time the swabs were in place.

Moore swabs were prepared by wrapping sterile cotton gauze, six inches wide by four feet long (15 cm by 120 cm), around a stiff wire. This was attached to a nylon cord and placed directly into the draining sewage. Most swabs were placed on Friday and collected on Monday to help ensure use of the facilities by the carriers in the households. After 48 to 72 h, each swab was removed from the sewage and placed directly into a wide-mouth jar containing 500 ml of Selenite-F broth (BBL Microbiology Systems, Cockeysville, Md.).

The swabs in the Selenite were incubated at 41°C and subcultured between 18 and 24 h onto Salmonella Shigella, bismuth sulfite [WB]), and DCLS (desoxycholate citrate lactose sucrose) agar. (All broth and media were from BBL.) Subculturing was done directly from the broth as well as with a 10-fold dilution of the broth. At 24 h after the swab was removed, the Selenite broth was again subcultured directly and with a 10-fold dilution on the same solid media. Suspicious colonies from the solid media were placed in TSI agar slants. Those giving TSI reactions typical of *S. typhi* were confirmed with standard biochemical tests and by agglutination with appropriate antiserum (3). All isolates were then phage typed.

The homes of 10 asymptomatic, chronic carriers of *S. typhi* were visited. No carrier was taking antibiotics. At least two swabs were placed at different times in each of the sewers draining the homes of these carriers. Table 1 lists the households, the number of swabs placed in each sewer, and the number of times the cultures were positive for *S. typhi*. A total of 24 swabs were placed, and of these, 6 were positive (25%). Although only 25% of the swabs were positive, 5 of the 10 carrier households (50%) were found to have culture-positive swabs.

WB was the most effective medium for isolation of *S. typhi* from the swabs. In four of the six isolates, WB was the only medium on which *S. typhi* could be identified. In one case, an isolate was recovered from both Salmonella Shigella

* Corresponding author.

TABLE 1. Number of swabs positive for *S. typhi* from chronic typhoid carriers

Carrier household	Isolation of *S. typhi* by Moore swab	No. of positive swabs/no. of swabs placed in sewer
1	−	0/2
2	−	0/3
3	−	2/2
4	−	1/3
5	−	0/3
6	−	1/3
7	−	1/2
8	−	1/2
9	−	0/2
10	−	0/2

medium and WB, and in only one instance was *S. typhi* isolated from Salmonella Shigella and DCLS media without recovery on WB. Of the six isolates, four were recovered on the first subculture (18 to 24 h), both directly and in the 1/10 dilution. Only one isolate that was not recovered by direct isolation was recovered at the 1/10 dilution.

In this study, we placed Moore swabs into the small-diameter sewers draining the homes of known, chronic typhoid carriers in Santiago, Chile. When two or three swabs were placed over time in each sewer, we were able to successfully recover *S. typhi* from one-quarter of the swabs and one-half of the carriers. The ability to isolate typhoid bacillus from these sewers seems to increase with increasing numbers of swabs. We suspect that as more swabs are placed, the ability to find a positive one for each carrier increases. Since we had no way of confirming that the carrier in the household was shedding typhoid bacilli during the time the swab was in place, this isolation rate probably represents a low estimate of the true sensitivity of the swab.

In studies in England in 1954, Kwantes and Speedy (5), while investigating a paratyphoid outbreak with Moore swabs, found that carriers tried to avoid using the toilet facilities to escape detection. We do not know if the typhoid carriers in the households we sampled avoided using the toilet during our swabbing. Ideally, we would have preferred to have simultaneous stool cultures with swab cultures to correlate sensitivity, but due to the study design that was not possible. Even so, our finding that the Moore swab was successful in identifying *S. typhi* carriers 50% of the time suggests that in field epidemiologic situations, it is a useful and practical tool.

In a previous study of Moore swabs in Chile, Sears et al. were able to isolate *S. typhi* 11% of the time from fecally polluted irrigation canals (9). Moore swabs proved to be reliable, inexpensive epidemiologic tools for the isolation of *S. typhi* in Chile, an endemic area. In this study, we have sought to refine our previous observations and have attempted to determine the crude sensitivity of the Moore swab in a field situation. Moore swabs will detect a known carrier at least 50% of the time if small sewers are sampled at least two separate times. Thus, the Moore swab is a reasonably sensitive method to isolate *S. typhi* and may have practical applications such as sampling the small sewers draining restaurants, food-processing plants, markets, or other institutions in which it could be important to detect carriers.

Moore, in his original studies, suggested that the sewer draining a block of homes was the ideal size for isolating *S. typhi* (8). We have taken this observation one step further and have shown that small sewers directly draining the homes of carriers can be sampled effectively for *S. typhi*. Our observations also reconfirm the utility of WB, as well as Selenite broth enrichment for isolating *S. typhi* and suggest that the use of WB alone may be sufficient since we would have missed only one isolate with such solitary use.

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Duodenal String Cultures: Practicality and Sensitivity for Diagnosing Enteric Fever in Children

The diagnosis of enteric (typhoid or paratyphoid) fever must be confirmed by isolation of the causative organism from a suitable clinical culture. This verifies the appropriateness of antibiotic therapy, allows differentiation between *Salmonella typhi* and *Salmonella paratyphi* A, B, and C infections and provides isolates for phage typing if epidemiological investigations are indicated. Bone marrow culture, the most sensitive method to recover organisms associated with enteric fever [1–7], requires special instruments and technical expertise and is uncomfortable and invasive; it is therefore not amenable to routine use in children with suspected enteric fever. Blood culture, in contrast, is widely practiced wherever bacteriology is available, because of its relative simplicity, safety, and noninvasiveness. Unfortunately, the sensitivity of blood culture is significantly less than that of bone marrow culture [1–7]. Since *Salmonella* are present in the bile of patients with acute enteric fever [8–10], some investigators have cultured bile-containing duodenal fluid by means of string capsule devices [7, 11–13] and have reported significantly higher rates of isolation of *Salmonella* than when cultures of blood are used alone. With one exception [14], these studies have been largely confined to adults. However, in endemic areas such as Santiago, Chile, typhoid fever is predominantly a disease of school-age children, five to 14 years of age [15]. Thus we undertook this study to evaluate the practicality and clinical acceptability of cultures of duodenal string capsule in children <15 years of age with a clinical diagnosis of enteric fever and to compare the sensitivity of this culture method with that of cultures of blood and bone marrow.

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Patients and Methods

Patients with a clinical diagnosis of acute enteric fever who were admitted to the Roberto del Rio Children's Hospital between January 1983 and February 1985 entered the study. From each child, an attempt was made to obtain blood, bone marrow, and bile-stained duodenal fluid for culture. Two 5-ml samples of blood, drawn 30 min apart by sterile technique, were inoculated into flasks containing 50 ml of brain-heart infusion broth (BBL Microbiology Systems, Cockeysville, Md) with 0.025% sodium polyanetholsulfonate. Aspirates of bone marrow from the iliac crest were inoculated into identical flasks.

To obtain samples of bile-containing duodenal fluid for culture, we instructed the children to swallow a string capsule device (Pediatric Enterotest®; HEDECO, Mountain View, Calif) with a glass of water or flavored gelatin. The device consists of a nylon string coiled within a gelatin capsule. The proximal end of the string was taped to the cheek, and the string was left in place for 6 hr. (During this time the gelatin capsule digests in the stomach and allows the nylon string to uncoil and pass through the pylorus into the duodenum, where the distal end is impregnated with bile and duodenal fluid.)

When the string was removed, the distal portion was examined for bile staining, and the pH was measured to determine whether the string had reached the duodenum. The distal 20 cm were severed and divided into two equal portions, one of which was inoculated into 20 ml of selenite F enrichment broth and the other into 50 ml of brain-heart infusion broth. Flasks were incubated at 35 C, and positive broths were subcultured onto salmonella-shigella and bismuth-sulfite agar (BBL). Suspicious colonies were transferred to Kligler's triple-sugar iron agar and characterized by standard biochemical and serological techniques. Chloramphenicol treatment was initiated (50 mg/kg per day) after all cultures were obtained.

Results

A complete set of cultures (two blood, one bone marrow, and one duodenal string) was obtained from 103 children,

three to 14 years of age, with a clinical syndrome compatible with typhoid fever. From the last 23 patients a second duodenal string culture was routinely obtained on the day following the initial cultures. Only three additional children attempted to swallow the string capsule device but were unsuccessful. Four children had received an antibiotic before admission.

Sensitivity of culture combinations. One of the three cultures was positive in all 103 clinically suspected cases, of whom 88 had *S. typhi* and 15 had *S. paratyphi* A, B, or C infection. The sensitivity of a single culture in bacteriologically confirming cases ranged from 61% for blood to 76% for bone marrow, with a single duodenal string culture as the intermediate (71%; table 1). Combinations of cultures greatly increased the sensitivity (table 1). A second culture of blood resulted in isolation of *Salmonella* from 69% of the cases, whereas adding a bone marrow culture to two cultures of blood increased the sensitivity to 84%. A single duodenal string culture in conjunction with two cultures of blood also notably increased sensitivity, a combination resulting in bacteriologic confirmation of 95 (92%) of 103 cases. Thus either a single bone marrow or a single duodenal string culture significantly increased the rate of bacteriologic confirmation over two cultures of blood alone. By means of two cultures of blood, one bone marrow culture, and one duodenal string culture, 101 of 103 patients were confirmed bacteriologically as having enteric fever (the remaining two patients were confirmed by means of a second duodenal string culture). The last 23 patients in this study had two duodenal string cultures routinely performed with isolation of *Salmonella* from 21 (91%) of 23 children by means of these two cultures.

Practicality, acceptability, and reliability of duodenal fluid cultures by the string-capsule device. Among the 103 children from whom duodenal cultures were obtained, eight children (8%) had notable difficulty in swallowing the capsule; a few had to be given a second capsule. No adverse effects were noted from the use of the string-capsule devices.

The pH of the distal tip of the string was recorded in 99 of the 103 children who had duodenal string cultures. The recovery of *Salmonella* from duodenal string cultures was clearly related to whether the string had passed through the pylorus into the duodenum (based on the pH of the tip of the string). Of 76 children whose strings had a pH ≥6.0, 59 (78%) had positive cultures. In contrast, when the pH was <6.0, only 10 (43%) of 23 yielded *Salmonella* ($P = .0042$); the pH in the 10 with positive cultures was 5.0 and was ≤4.0 in those with negative cultures. The rate of positive duodenal string cultures did not differ significantly in relation to age; 27 (79%) of 34 children three to nine years of age had positive cultures vs. 46 (67%) of 69 children 10 to 14 years of age ($P = .70$).

The rate of positivity of duodenal string cultures in relation to duration of illness before entering the study is shown in table 2. The duration of illness was not significantly different for the 73 children with positive cultures vs. the 30 children with negative cultures (table 2).

Table 1. Comparison of the relative efficacy of cultures of blood, bone marrow, and duodenal string, singly or in combination in isolating *S. typhi* or *S. paratyphi*.

Type of culture	No. of positive cultures (%)
Single	
1st blood	61 (59)
2nd blood	63 (61)
duodenal string	73 (71)
bone marrow	78 (76)
Combinations	
2 blood	71 (69)
2 blood + 1 bone marrow	87 (84)
2 blood + 1 duodenal string	95 (92)
2 blood + 1 bone marrow + 1 duodenal string	101 (98)

NOTE. Cultures were obtained from 103 children 3–13 years of age with clinical enteric fever.

Table 2. Positivity of duodenal string cultures in children with enteric fever in relation to the duration of illness.

Days of illness before culture	No. of children	No. positive/no. negative (% positive)
1–7	26	17/9 (65.4)
8–14	48	38/10 (79.1)
15–21	26	16/10 (61.5)
22–31	3	2/1 (67)
Total	103	73/30
Median*	–	10/10
Mean ± SD*	–	12.0 ± 6.4/10.0 ± 4.3

NOTE. The duration of illness was not significantly different in those with positive vs. those with negative cultures (Student's *t* and Wilcoxon rank sum tests).

* Data are days of illness before culture in positives/days of illness before culture in negatives.

Discussion

Other investigators have previously documented the usefulness and sensitivity of duodenal string cultures in typhoid fever in studies largely involving adults [7, 13]. Benavente et al. [13] found duodenal string cultures positive in 86% and positive cultures of bone marrow in 75% of 36 Peruvians with typhoid fever. Hoffman et al. [7] reported that the combination of one culture of blood, one rectal swab, and one duodenal string culture had 86% sensitivity vs. 92% sensitivity for the combination of one

culture of blood, one rectal swab, and one bone marrow culture in isolating *S. typhi* or *S. paratyphi* from 118 Indonesians with enteric fever. The only previous pediatric study involved 118 Peruvian patients two to 13 years of age with suspected enteric fever [14] who swallowed "homemade" string-capsule devices prepared locally at the hospital. Only 47% of 38 young Peruvian children two to six years of age tolerated the duodenal string cultures; furthermore, the sensitivity of the duodenal string cultures was much lower than that previously reported for adult patients at that hospital in Peru [12, 13].

Herein we report a systematic study of duodenal string cultures in comparison with cultures of bone marrow and blood in 103 Chilean children (14 years of age or younger) with a clinical diagnosis of enteric fever. A single duodenal string culture, in conjunction with two cultures of blood, allowed isolation of *S. typhi* or *S. paratyphi* from 92% of patients, a comparable rate occurred with two cultures of blood and a bone marrow culture (84%). The string capsule device was practical and surprisingly well tolerated by the children; 103 (97%) of 106 children who attempted succeeded in swallowing the string capsule, with 98 (92%) having no notable difficulty whatsoever. Furthermore, *Salmonella* were as readily isolated from duodenal string cultures in young children three to nine years of age (27 [79%] of 34) as older children ≥10 years of age (46 [67%] of 69; $P = .70$). The recovery of *Salmonella* from string cultures correlated highly with evidence (by measurement of the pH of the distal end) that the string had reached the duodenum; when the strings had a pH ≥6.0, *Salmonella* was recovered from 78% of the cultures vs. only 43% when the string pH was <6.0 ($P = .0042$). Our results in Chilean children contrast sharply with those in Peruvian children [14], with both clinical acceptability and sensitivity being significantly greater in our study. The two studies differ so markedly in methods, however, that caution must be exercised in making comparisons. The Peruvian study utilized homemade rather than commercial string devices, and it was not stated if these were modified for pediatric patients. Furthermore, the Peruvian investigators removed the strings after 3 hr and most importantly, did not verify the pH of the tip of the string. The high sensitivity in the Chilean study may be due, in part, to the strings being left in place for an average of 6 hr; future comparative studies will assess if 3 or 4 hr will suffice, thereby making duodenal string cultures more practical for outpatients.

In the last 23 patients a second duodenal string culture was routinely obtained, thereby allowing us to make a preliminary statement of the value of two duodenal string cultures. Among these 23 patients, at least one of the duodenal string cultures was positive in 21 patients (91%), vs. two cultures of blood yielding a *Salmonella* in only 14 (61%) cases and two cultures of blood plus a bone marrow culture confirming 18 (78%) cases of enteric fever. If two duodenal string cultures are desired without unduly delaying the initiation of antibiotics, it may be prudent to obtain the cultures one immediately after the other.

In children, culture methods to confirm the diagnosis of enteric fever must compromise between sensitivity and practicality. Two cultures of blood represent the minimum effort to be expected wherever bacteriologic capability is available, since they are simple to obtain and noninvasive. Unfortunately, cultures of blood offer only moderate sensitivity. Sensitivity can be notably increased if clinical material can be obtained for culture from the reticuloendothelial system where *Salmonella* reside in specific macrophages. Heretofore, this has been accomplished by means of bone marrow cultures. This procedure, however, is invasive for children and requires skilled operators and special needles that are not always available.

Our systematic study in Chilean children with enteric fever demonstrates that the combination of two cultures of blood and a duodenal string culture offers excellent sensitivity (equal to two cultures of blood and a bone marrow culture) and noninvasive practicality and is effective in children from three to 14 years of age.

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Phenotypic Characterization of *Neisseria gonorrhoeae*

Meningitis is a rare complication of disseminated gonococcal infection (DGI). Although DGI has been estimated to occur in 1%–3% [1] of all gonococcal infections, fewer than 30 cases have been reported in the past twenty years [1–3]. In [illegible] serological and phenotypic studies, we describe the gonococci isolated from three cases that occurred in Philadelphia between January and July 1984.

Case Summaries

Case 1. A 15-year-old black girl was hospitalized with a 24-hr prodrome of malaise, sore throat, migratory joint pains in her wrists and ankles, purpuric skin lesions, vomiting, and [illegible] confusion. The patient was neither pregnant nor menstruating. A gram stain of the smear of the CSF revealed no organisms, and bacterial cultures were sterile. An [illegible] CSF neutrophilic leukocytosis was noted, however, along with depressed levels of glucose and elevated levels of protein. Cultures of blood and cervix were positive for *Neisseria gonorrhoeae*. Despite aggressive management including parenteral antimicrobial therapy using penicillin and chloramphenicol, the patient died of overwhelming sepsis [4].

Case 2. A 19-year-old black woman was admitted to a second hospital with a three-day history of migratory

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ises of Meningitis

panied by nausea, vomiting, The patient was neither preg- ination of the CSF revealed depressed levels of glucose, . A gram stain of the smear bacteria. CSF and cervical *gonorrhoeae*. The patient microbial therapy that in- charged without serious se-

eck man was admitted to a h a diagnosis of a partially mation was consistent with obtained at another hospi- *hoeae*. The patient respond- l therapy without sequelae.

Isolates from the three pa- ures of blood, CSF, and cer- *hoeae* by carbohydrate utili- edures [5]. β-Lactamase the chromogenic cephalo- re obtained from synovial 10 other patients who had meningitis. A total of 50 ndomly selected from pa- unity with a diagnosis of with no apparent compli- irmed as *N. gonorrhoeae* ctivity.

nococci from all patients, . were serologically classi- fic monoclonal antibod- cal outer membrane in a

APPENDIX M

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Survey of Plasmids in *Salmonella typhi* from Chile and Thailand

Salmonella typhi remains an important enteric pathogen in many parts of the world. Although a number of outbreaks of typhoid fever have been caused by antibiotic-resistant *S. typhi*, such as in Mexico in the early 1970s [1], and more recently in Peru, these organisms have in general remained surprisingly susceptible to antibiotics, particularly when one compares their resistance with that of other enteric pathogens, like the shigellae and nontyphoidal salmonellae. The current study was originally undertaken to investigate antibiotic resistance in *S. typhi* in Santiago, Chile and to examine total plasmid content of clinical isolates. This study also explored the possibility of a common "virulence" plasmid(s) and the potential utility of plasmid electropherotyping for epidemiological studies. When no resistance and few plasmids were found, further studies were undertaken to investigate possible reasons for these findings and to determine if similar results could be found in other geographic locations.

Materials and Methods

Bacterial strains and susceptibility testing. Clinical isolates of *S. typhi* from local hospitals in Chile were identified and phage typed at the Instituto de Salud Pública in Santiago; strains from Thailand were sent to the Department of Medical Science, Bangkok. Recipient strains included *Escherichia coli* J53 (*pro met*) and nalidixic acid–resistant mutants of three plasmid-free *S. typhi* clinical isolates from Chile. Donor strains were *E. coli* isolated either from urinary tract infections in Santiago, or from feces of a U.S. student in Mexico [2]. Antimicrobial susceptibilities were determined by the disk-diffusion method by using Mueller-Hinton agar (Difco Laboratories, Detroit) and disks purchased from BBL Microbiology Systems (Cockeysville, Md).

Conjugal transfer and plasmid studies. Total plasmid contents of all strains were examined by the method of Kado and Liu [3]; a subset of 30 strains from Chile were also cross-examined by other methods [4, 5]. Conjugations were performed in broth as previously described [2, 5]. The frequency of transfer was determined by dividing the number of transconjugants by the number of recipients.

Growth curves and stability studies. For growth rate determinations, single colonies were inoculated in duplicate into brain-heart infusion broth, grown overnight at 37 C, diluted 10^{-4} into brain-heart infusion broth and incubated in a rotary incubator at 200 rpm at 37 C. Growth was followed at 580 nm with a Spectronic 21® (Beckman Instruments, Palo Alto, Calif). The stability of plasmids was determined by inoculating 10 single colonies of each strain from trimethoprim-containing agar plates onto peptone agar slants; after incubation at 25 C for 3 months, each slant was subcultured and 8–12 single colonies from each slant (80–100 total per strain) were tested for resistance.

Results

Chilean strains. One hundred strains of *S. typhi* isolated within the preceding year in Chile were examined; 19 were examined within one week of isolation. Phage typing of 74 isolates revealed that the majority of strains were either type E1 (23 isolates), type 46 (17 isolates), or F8 (8 isolates); other types included F1 and M1 (4 strains each), A and 34 (3 strains each), 38 (2), D4 (1), nontypable (2), and Vi(−), (7). None of these isolates were resistant to any of seven antimicrobial agents tested; this corroborates the results of D'Ottone et al. [5] in Chile in 1980 that showed only two of 661 isolates were resistant to chloramphenicol and the results of Rodriguez *et al.* [7] in 1977 that showed only 1.8% of 1,622 isolates were resistant to any of the six agents.

Of 100 Chilean isolates that were examined for the presence of extrachromosomal DNA, only eight were found to have plasmids; all eight were detected by the method of Kado and Liu. Five phage type F8 isolates and one Vi(−) isolate had a plasmid of 65 Mdal; one type 38 strain had a plasmid of 32 Mdal, and one nontypable strain had a plasmid of 3 Mdal.

Thai strains. Since Chile is somewhat isolated geographically, strains of *S. typhi* from another location were examined. Fifty strains from Thailand were screened by phage typing, and 38 revealed the following: type 46 (9 strains), type M1 (7), type E1 (6), type D1 (3), type 53 (2), types E9, J5, D6, and D5 (1 strain each), Vi(−) (2), and 5 strains were untypable. Three were found to have plasmids. One of these three strains (phage type D1) was resistant to ampicillin, chloramphenicol, streptomycin, and tetracycline. Another isolate was resistant by disk to streptomycin (9 mm zone of inhibition), and ten were intermediate in susceptibility to streptomycin; none of these had a plasmid.

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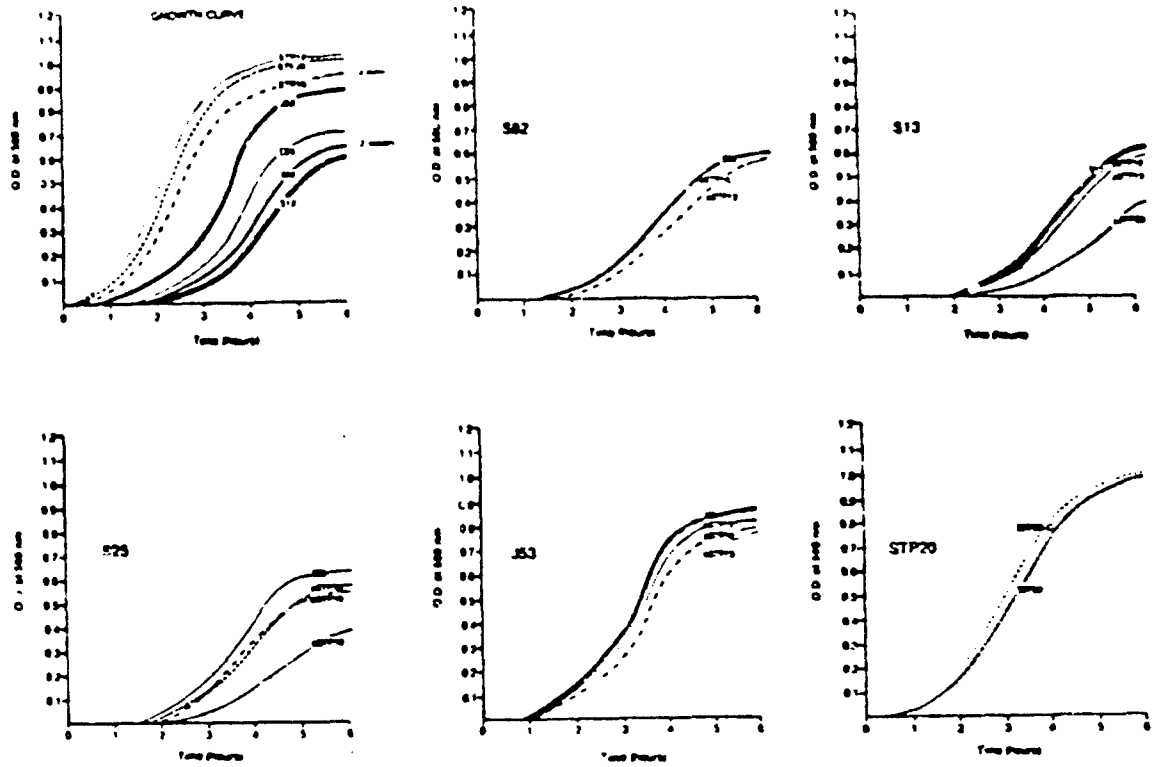

Figure 1. Experiments to determine growth curves of parental strains and their plasmid-containing derivatives were performed in brain-heart infusion broth. Experiments were performed with STP12, STP13, STP18, and STP20 *Escherichia coli* isolates from urinary tract infections in Chile; pSTP12, pSTP13, pSTP18, and pSTP20 are the resistance plasmids derived from the corresponding strains. S13, S25, and S82 are *Salmonella typhi* isolates from Chile. The upper-left figure shows the growth curves of all strains before transferring any R factors. The next four figures show *S. typhi* strains without (solid lines) and with (broken lines) various R factors. The lower-right figure shows STP20 and a spontaneously arising derivative (STP20-C), which has lost resistance.

trimethoprim-sulfamethoxazole) are available over-the-counter in these as in most developing countries. Chloramphenicol, for example, considered the primary agent for typhoid fever, has been widely used for various illnesses since the 1950s. Studies of other enteric organisms that cause diseases and are treated with similar antibiotics, such as *Salmonella newport* and *S. typhimurium* in Chile and *Salmonella krefeld* in Bangkok, reveal multiple antibiotic resistances [12, 13; P. J., unpublished data]. Surveillance of *E. coli* in Santiago and Bangkok has revealed increased resistance in this species [14].

In order to investigate the interaction of *S. typhi* and several R factors found in nature, we performed conjugation studies between clinical isolates of *S. typhi* and *E. coli*. *E. coli* seems a likely donor species in nature for the following reasons: (*1*) it is normally the most numerous coliform in the human intestinal tract and therefore should come into contact with *S. typhi* (2) in developing countries it is often multiply resistant; and (*3*) since both *E. coli* and *S. typhi* are Enterobacteriaceae and considerably homologous by DNA studies, they could be expected to exchange genetic information in vivo. The transfer frequencies of the R factors originating in *E. coli* into *S. typhi* were, at most, slightly decreased relative to an *E. coli* recipient (table 1). This implies that neither a restriction barrier nor a restriction endonuclease impedes the entry or establishment of *E. coli* plasmid DNA in *S. typhi*.

As expected, a comparison of growth rates revealed that the clinical *E. coli* isolates grew more rapidly than did the laboratory K12 strain and much more rapidly than did the *S. typhi*. The presence of some but not all R factors further slowed the growth rates of both *E. coli* J53 and of *S. typhi*, but a consistent effect of a given plasmid upon all host strains was not seen. A slowing effect has been well documented for some plasmids, although some have no effect and some even enhance growth of the organism [15, 16]. Whether the slowing effect on *S. typhi* growth seen with some of these R factors would impart a selective disadvantage in nature is unknown.

Another, perhaps more important, difference between the *E. coli* and *S. typhi* hosts was the degree of stability of the R factors. Four of the R factors were unstable in *S. typhi* but stable in *E. coli* (table 1). Such instability suggests that a number of accessible R factors in nature do

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Disproportionate Expansion of a Minor T Cell Subset in Patients with Lymphadenopathy Syndrome and Acquired Immunodeficiency Syndrome

The evolution of acquired immunodeficiency syndrome (AIDS) involves alterations in lymphocyte subpopulations that may be a significant part of the underlying disease process. These alterations in lymphocyte subsets include an inversion of the T4:T8 ratio that is due to a reduction in the absolute numbers of T4 positive cells and either normal numbers or slightly elevated numbers of T8 positive cells [1]. As the disease progresses, lymphopenia results in lower absolute numbers of both $T4^+$ and $T8^+$ cells and the T4:T8 ratio becomes even more reduced.

In viral infections with such viruses as cytomegalovirus (CMV), herpesvirus, or Epstein-Barr virus (EBV) a reversal in the ratio of $T4^+$ to $T8^+$ cells also occurs, and the effect may persist for months after recovery [2]. The reversal in these viral infections is primarily due to a dramatic expansion of the $T8^+$ population, although a reduction in the numbers of $T4^+$ cells does occur. Thus, in AIDS the reversal of the T4:T8 ratio reflects a somewhat different absolute representation of these T cell subpopulations than is observed in other viral infections, although the effect on the relative proportion of $T4^+$ and $T8^+$ cells may be similar.

In contrast to the findings in patients with AIDS and acute viral infections, Kornfeld et al. [3] showed that healthy, promiscuous homosexual men had increased numbers of $T8^+$ cells and normal numbers of $T4^+$ cells. A similar observation was made by Lederman et al. [4] in hemophiliacs who had received lyophilized preparations of antihemophilic factor. In patients with the AIDS-related complex (ARC) of symptoms and physical findings, both an expansion of the $T8^+$ population and a reduction in the $T4^+$ lymphocyte subset have been observed [3, 5]. Because patients with ARC have a greater risk of developing AIDS, it is crucial to determine the temporal relationship of these lymphocytic alterations and their relevance to the eventual progression to AIDS.

We therefore examined patients with AIDS and ARC to determine whether the profound immunosuppression seen in these patients may be associated with more specific alterations in suppressor T lymphocyte subpopulations. We found that AIDS patients and ARC patients differed significantly from normal subjects and from individuals suffering acute viral infections. These differences included an increase in subpopulations of $T8^+$ cells bearing an additional cell surface determinant, Leu7, and an increase in numbers of $Leu11^+$ cells. This finding was in marked contrast to the relative infrequency of $T8^+Leu7^+$ cells in normal subjects [6], in which these cells constitute a minor subpopulation of $T8^+$ cells. Furthermore, the data suggest that evolution of the immunodeficient state may include an expansion of the $T8^+Leu7^+$ subpopulation in those patients with ARC who progress to the development of AIDS. When lymphopenia develops in AIDS patients, all subpopulations of lymphocytes are depleted and eventually only $T8^+Leu7^+$ cells remain. These alterations in lymphocyte subpopulations may provide new clues to understanding the pathogenesis of AIDS.

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Informed consent was obtained from all patients and normal volunteers according to the guidelines for human experimentation at Baylor College of Medicine.

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Subjects and Methods

Subjects. Patients with AIDS, ARC, and viral infections were referred for cell surface phenotyping to the Howard Hughes Medical Institute flow cytometry facility. All patients with AIDS were diagnosed according to

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MOLECULAR TECHNIQUES IN THE STUDY OF *SALMONELLA TYPHI* IN EPIDEMIOLOGIC STUDIES IN ENDEMIC AREAS: COMPARISON WITH Vi PHAGE TYPING

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Abstract. We examined 141 *Salmonella typhi* strains of known phage type isolated during ongoing epidemiologic studies in Santiago, Chile, and Lima, Peru. Plasmids were present in 12 (17%) of 70 *S. typhi* isolates from Santiago and 5 (7%) of 71 isolates from Lima; these plasmids were not associated with antimicrobial resistance. Identical 21 kilobase (kb) plasmids (as defined by restriction endonuclease digest pattern) were present in 13 of the 17 plasmid-containing isolates. Virtually identical digest patterns were identified when chromosomal DNA of selected strains from Santiago, Lima, and the United States was extracted and then digested with restriction endonucleases. The similarities among plasmids and chromosomal digest patterns emphasize the homogeneity and possible clonal origin of *S. typhi* isolates; these data also suggest that there is only a limited role for plasmid and chromosomal analysis as a substitute for phage typing in epidemiologic studies.

Typhoid fever is a major urban health problem along the western coast of South America, with reported incidence rates of 150 and 212 cases/100,000 in Santiago and Lima, respectively.[1,2] Studies of the epidemiology of typhoid fever in these areas are notoriously difficult because of the multiplicity of vehicles and risk factors present.[3,4] The ability to differentiate strains based on specific epidemiologic markers is critical in such studies; unfortunately, few markers for *Salmonella typhi* have been described, with Vi phage typing currently providing the only useful means of distinguishing one *S. typhi* strain from another. Molecular genetic techniques, including plasmid analysis and examination of restriction endonuclease digest patterns of chromosomal DNA, have been found to be valuable tools in epidemiologic studies of certain other bacterial pathogens.[5–7] We studied selected isolates from Chile and Peru to determine if these molecular techniques were useful in differentiating *S. typhi* strains beyond what could be accomplished with Vi phage typing alone.

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MATERIALS AND METHODS

S. typhi strains from Chile were randomly selected from strains isolated from pediatric patients seen at the Roberto Del Rio Hospital in Area Norte, Santiago, between January and June 1983. The identification of the isolates was confirmed by the Institute of Public Health, Santiago, and isolates were phage typed by the Vi phage typing scheme of Anderson and Williams[8] at the Institute of Public Health, Santiago, and the Division of Enteric Pathogens, Central Public Health Laboratory, Colindale, England. Strains from Lima were isolated from pediatric and adult patients between February and December 1984 at the Universidad Peruana Cayetano Heredia, Lima;* isolates were phage typed at the Central Public Health Laboratories, Colindale. American *S. typhi* strains were isolated from adult patients in Maryland and Texas.

Plasmids were extracted from isolates using an alkaline extraction procedure.[10] All strains containing plasmids were tested by disc diffusion for susceptibility to ampicillin, chloramphenicol, gentamicin, and trimethoprim/sulfamethoxazole. Chromosomal DNA was extracted from

TABLE 1

Number of isolates with specific plasmids, and total number of isolates, by Vi phage type and area of isolation

Phage type	Plasmid size			No plasmids	Total (%)
	21 kb	29 kb	57 kb		
Chile					
E_1	–	–	–	30	30 (43%)
46	2	–	–	16	18 (26%)
51	4	–	–	2	6 (9%)
M_1	–	–	–	2	2 (3%)
F_6	1	–	–	1	2 (3%)
34	1	–	–	1	2 (3%)
D_4	–	–	1	1	2 (3%)
38	1	–	–	–	1 (1%)
A	–	–	–	1	1 (1%)
D_1	–	–	–	1	1 (1%)
F_1	–	–	–	1	1 (1%)
Vi neg.	1	–	1	1	3 (4%)
Untypable	–	–	–	1	1 (1%)
Total	10	0	2	58	70 (100%)
Peru					
M_1	–	–	–	17	17 (24%)
46	–	–	–	10	10 (14%)
A	–	–	–	8	8 (11%)
35	3	–	–	4	7 (10%)
E_1	–	–	–	4	4 (6%)
29	–	–	1	3	4 (6%)
B_2	–	–	–	4	4 (6%)
H	–	–	–	1	1 (1%)
26	–	–	–	1	1 (1%)
Vi neg.	–	–	–	2	2 (3%)
Untypable	–	–	–	7	7 (10%)
Degraded Vi	–	1	–	5	6 (8%)
Total	3	1	1	66	71 (100%)

isolates using a phenol/chloroform extraction procedure.[11] DNA was digested with restriction enzymes (*Eco*RI, *Hind*III, *Bam*HI, or *Pvu*II; Bethesda Research Laboratories, Inc.), and visualized under ultraviolet light after electrophoresis in 0.7% agarose gels and staining with ethidium bromide.

RESULTS

Eleven phage types were represented among 70 *S. typhi* strains isolated from patients in Santiago. Vi phage type E1 accounted for 43% and Vi phage type 46, 26% of isolates (Table 1). Plasmids were present in 12 (17%) of the 70 strains. Two distinct plasmid profiles were identified: 10 isolates had a single 21 kilobase (kb) plasmid and 2, a 57 kb plasmid. Isolates with the 21 kb plasmid were significantly more likely to be of Vi phage type 51, with four of six isolates of this phage type carrying the plasmid ($P < 0.01$, Fisher's exact test, two-tail). All plasmid-carrying strains were susceptible to the four antimicrobial agents tested. No correlation could be shown between specific plasmid profiles and time or place of isolation of the strain, or age or sex of the patient from whom the strain was isolated.

Nine phage types were represented among the 71 *S. typhi* strains isolated from patients in Lima. Vi phage type M1 accounted for 30% and Vi phage type 46 18% of typable isolates (Table 1). Plasmids were present in 5 (7%) of the 71 strains. Three plasmid profiles were identified among the Peru isolates: 3 isolates had a single 21 kb plasmid, 1 a 57 kb plasmid, and 1 a 29 kb plasmid. Presence of the 21 kb plasmid was significantly associated with Vi phage type 35, with 3 of 7 isolates of this phage type carrying the plasmid ($P < 0.01$; no isolates of Vi phage type 51 were identified among the Peru isolates. All strains carrying plasmids were susceptible to antimicrobial agents tested. When cut with each of three

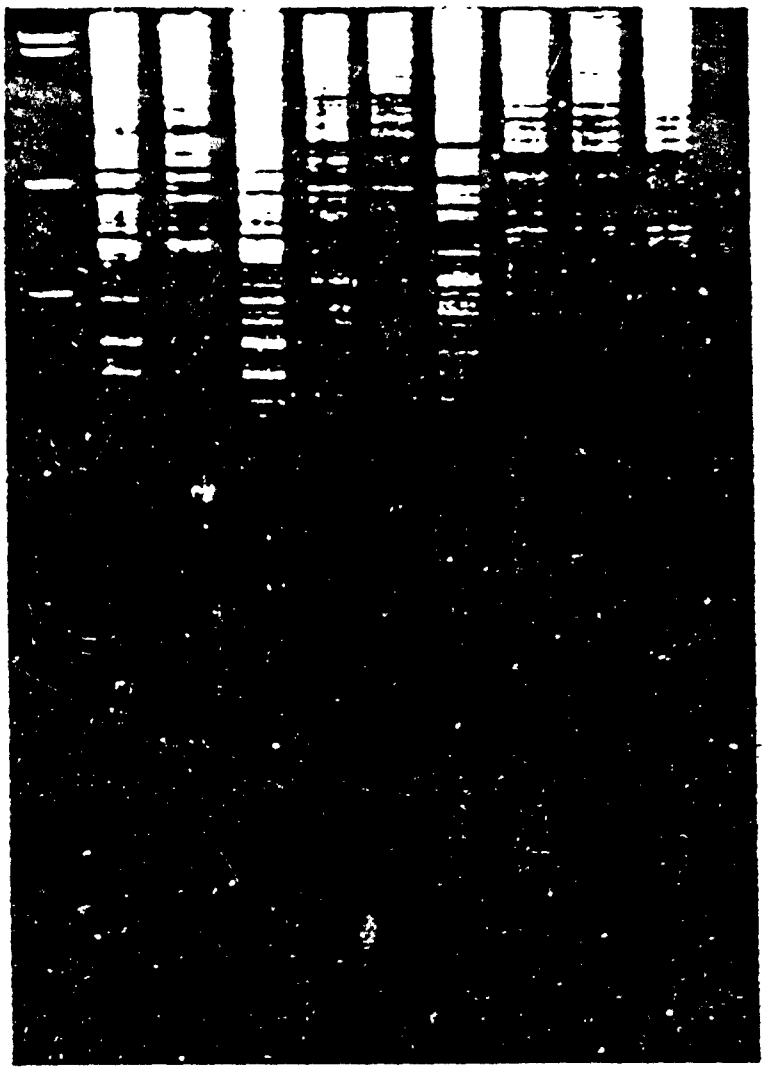

FIGURE 1. Chromosomal restriction endonuclease digests of selected *S. typhi* isolates: a. Lambda *Hind*III; b. Isolate A, Chile, *Hind*III digest; c. Isolate B, Peru, *Hind*III digest; d. Isolate C, U.S., *Hind*III digest; e. Isolate A, Chile, *Eco*RI digest; f. Isolate B, Peru, *Eco*RI digest; g. Isolate C, U.S., *Eco*RI digest; h. Isolate A, Chile, *Pvu*II digest; i. Isolate B, Peru, *Pvu*II digest; j. Isolate C, U.S., *Pvu*II digest.

restriction endonucleases (*Hind*III, *Eco*Ri, or *Bam*HI) 21 kb plasmids identified in both Lima and Santiago had identical restriction fragments. Restriction fragments of the 57 kb plasmid identified in Santiago differed from those of the Lima 57 kb plasmid.

Chromosomal DNA was extracted from 32 *S. typhi* strains from Chile, 28 strains from Peru, and 3 strains from the United States. Restriction endonuclease digest profiles were identical for all strains after digestion with *Hind*III, *Eco*RI, or *Bam*HI. DNA from 17 of these strains (9 from Chile, 7 from Peru, and 1 from the United States) was also cut with *Pvu*II. After *Pvu*II digestion it was possible to identify 2 slightly different restriction profiles (Fig. 1). Both profiles were pres-

ent among strains from Chile and Peru, with no apparent correlation between either of the profiles and phage type or source of the isolate; both profiles were present among isolates having the same phage type.

DISCUSSION

We found that less than 20% of antibiotic-sensitive *S. typhi* strains in Santiago and Lima carried plasmids, in keeping with previous studies of *S. typhi* from these and other geographic areas.[12] The similarities in plasmid sizes among Santiago and Lima isolates prompted us to further characterize the plasmids based on restriction endonuclease digest pattern. While there were differences between the 57 kb plasmids present in the two cities, the 21 kb plasmids found in 13 of the 17 plasmid-containing strains appear to have been identical. These data emphasize the similarities among *S. typhi* strains in the two areas, and the apparent lack of diversity among plasmids not encoding resistance to antimicrobial agents. Similar observations have been made with *S. typhi* antimicrobial resistance plasmids of incompatability group H_1, with one study demonstrating that 8 resistant isolates from 4 different geographic areas either had identical plasmids, or had plasmids that were very similar based on sequence homology.[13] Limited in vitro studies suggest that this lack of diversity, at least among resistance plasmids, is the result of plasmid instability in *S. typhi*, rather than an inherent barrier to the entry or establishment of foreign plasmid DNA.[12]

In this study for the first time chromosomal restriction endonuclease digests of *S. typhi* strains were systematically examined. In contrast to observations made with other species,[5-7] the chromosomal patterns of our isolates were almost identical. We were able to demonstrate differences between strains with only 1 of the 4 restriction enzymes used; differences that were observed were minor, with only 2 different patterns noted among the isolates studied. In contrast, each of 4 *S. paratyphi* A strains from Lima studied at the same time had a distinct digest pattern (K. O'D. Maher, personal communication). Previous investigators have noted the striking biochemical and serological similarities among *S. typhi* strains isolated in different geographic areas, and proposed that *S. typhi* strains represent a "clone" that has retained a remarkable degree of homogeneity, despite worldwide distribution of the disease;[14] our observations support this concept.

While plasmid profiles may be of use in outbreak situations or in following transmission of a specific strain in a community (provided the strain carries a plasmid), our data make it clear that plasmid analysis cannot be a substitute for a general typing scheme such as phage typing. The association between plasmids and specific phage types is a further disadvantage from an epidemiologic viewpoint, with plasmid profiles providing little help in subdividing the major Vi phage groups such as E1. Similarly, chromosomal restriction endonuclease digests do not appear to be a useful epidemiologic tool for investigation of *S. typhi* outbreaks. However, further molecular studies, including studies of isolates from other geographic areas, may provide some insight into the observed lack of diversity among plasmids in antibiotic-sensitive *S. typhi* strains, and into the phylogeny and possible clonal origin of the organism.

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PROGRESS IN VACCINES AGAINST TYPHOID FEVER

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Running Head: New Typhoid Vaccines

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ABSTRACT

The widely available heat-phenol-inactivated whole cell typhoid vaccine, which provides approximately 65% protection, has limited usefulness because of the adverse reactions it evokes. In contrast, several new typhoid vaccines promise protection without reactogenicity. Attenuated oral vaccine Ty21a has been evaluated in three field trials of efficacy in Santiago, Chile, involving 450,000 schoolchildren. Three doses of Ty21a in an enteric-coated formulation given within one week provided 67% efficacy for at least three years. Less protection followed administration of fewer doses, while adding a fourth dose significantly enhanced protection; increasing the interval between doses did not improve protection. Large-scale vaccination with Ty21a appeared to cause a herd immunity effect. Ty21a has reached the stage of being a practical public health tool. Regarding other vaccines, the safety and immunogenicity of an auxotrophic (Aro-, Pur-) S. typhi mutant (strain 541Ty) has recently been demonstrated. Lastly, parenteral purified Vi polysaccharide of S. typhi, shown to be safe and immunogenic in young adults, is being evaluated for efficacy in controlled field trials.

INTRODUCTION

Typhoid fever remains an important public health problem in many less-developed regions of the world and poses a risk for travelers from industrialized countries who visit such endemic regions (1-4). In virtually all endemic areas the incidence rates for typhoid fever are highest in children 5-19 years of age, i.e. schoolchildren (5-9). This is of potential relevance in the control of typhoid, since schoolchildren represent a "captive" population amenable to school-based immunization programs.

Field Trials with Parenteral Killed Whole Cell Typhoid Vaccines

Parenteral killed whole cell typhoid vaccines, available since 1896 (10-12), have been used throughout this century. In the 1950s and 1960s the World Health Organization sponsored a series of large-scale field trials in several countries to assess the efficacy of various types of parenteral killed whole cell vaccines. In the first of these trials, in Yugoslavia, a fluid heat-inactivated, phenol-preserved parenteral vaccine was found to be superior in protective efficacy in comparison with an alcohol-inactivated and preserved vaccine (13-14).

Shortly after results of the above field trials in Yugoslavia became available, the Walter Reed Army Institute of Research in Washington, D.C. prepared for the World Health Organization two lyophilized reference vaccines for use in several additional field trials (15). These included a heat-phenol-inactivated and an acetone-inactivated vaccine, referred to respectively as L and K vaccines. The reference L and K vaccines were

evaluated together in randomized, controlled, double-blind trials in Yugoslavia and Guyana (16,17); in addition, the K vaccine was tested for efficacy in controlled trials in Poland and the L vaccine in the U.S.S.R. (18,19). Results of these trials are summarized in Table 1. While both reference vaccines conferred significant protection in the field trials in Yugoslavia and Guyana, the K vaccine was found to provide significantly superior protection than the L vaccine. In three separate trials, L vaccine conferred 51% (Yugoslavia), 66% (U.S.S.R.), and 67% (Guyana) protection (Table 1).

Although somewhat more efficacious, the acetone-inactivated vaccine is largely unavailable. Of the manufacturers of parenteral killed whole cell typhoid vaccines listed in the WHO's International List of Availability of Vaccines and Sera (21), 40 make the heat-phenol-inactivated variety while only three manufacture the lyophilized acetone-inactivated vaccine. Moreover, because of the high rates of adverse reaction that they elicit, parenteral killed whole cell typhoid vaccines are rarely used by any country in systematic typhoid fever control programs (with the possible exception of Thailand). A summary of the adverse reaction rates encountered in the WHO-sponsored field trials of K and L vaccines in Yugoslavia (16), Guyana (21), and the U.S.S.R. (19) are shown in Table 2.

Oral Killed Whole Cell Vaccines

It has been known for many decades that killed whole S. typhi can be safely given by the oral route without eliciting adverse reactions, in contrast with their reactogenicity when administered parenterally. However, in both experimental challenge studies in volunteers and in controlled field trials in endemic areas, killed whole cell vaccines given orally have provided little if any protective efficacy (22-26).

New Typhoid Vaccine Candidates

Several new candidate typhoid vaccines have emerged that offer the promise of significant protection without causing notable adverse reactions. These include two attenuated S. typhi strains used as live oral vaccines (strains Ty21a and 541Ty) and a purified subunit parenteral vaccine consisting of the Vi polysaccharide of S. typhi. The state of development of these vaccines is reviewed below.

DEVELOPMENT OF TY21A LIVE ATTENUATED ORAL VACCINE

Volunteer Studies with Ty21a

An important advance for the potential control of typhoid fever was the development by Germanier and Furer (27) of an attenuated strain of S. typhi, Ty21a, that can be utilized as a live oral vaccine. In preliminary studies in adult volunteers in North America, Ty21a was found to cause no adverse reactions, to be genetically stable, and to significantly protect against experimental infection with an inoculum of pathogenic S. typhi that caused typhoid fever in 53% of control volunteers (28).

Egyptian Field Trial of Ty21a

Based on these highly encouraging observations in adult volunteers, Ty21a vaccine was evaluated for efficacy by Wahdan et al (29,30) in a placebo-controlled, randomized, double-blind trial in Alexandria, Egypt. In this trial, three doses of Ty21a vaccine ($1-3 \times 10^9$ viable vaccine organisms per dose) or placebo were given to schoolchildren on Monday, Wednesday, and Friday of one week. Prior to ingestion of vaccine or placebo, children chewed a tablet containing 1.0 gm of $NaHCO_3$ (to neutralize gastric acid). Each dose of lyophilized vaccine or placebo was contained within glass vials in vacuo. The vials were opened, the lyophilate reconstituted in the field with diluent, and the liquid vaccine

(or placebo) suspension given to the child a few minutes after the child ingested the $NaHCO_3$ tablet. Passive surveillance failed to identify notable adverse reactions in the Egyptian schoolchildren, corroborating the safety of the live oral vaccine.

During the 36 month period of surveillance in Alexandria, the vaccine efficacy was 96% (Table 3) (29).

Field Trials of Ty21a in Santiago, Chile

Rationale

Shortly after the Egyptian field trial established the biological safety and efficacy of Ty21a in schoolage children in an endemic area, the Swiss Serum and Vaccine Institute made a formulation of vaccine commercially available which consisted of two gelatin capsules each containing 0.4 gm of $NaHCO_3$ and a third gelatin capsule containing lyophilized vaccine. Although this formulation resembled that used in Alexandria, Egypt, it was clearly not identical. Despite the highly encouraging results in the first field trial in Egypt, it was obvious that additional information had to be obtained before the Ty21a live oral vaccine could be employed as a practical public health tool. Some of the critical questions yet to be answered included:

1) What was the efficacy of Ty21a when administered in a formulation such as enteric-coated capsules that does not require pretreatment with $NaHCO_3$?

2) Could fewer doses (one or two) than used in Alexandria provide a satisfactory level of protection?

3) What level of protection would Ty21a provide in areas with incidence rates of typhoid fever much higher than the 44-50 cases/10^5/year that prevailed during the trial in Alexandria?

4) What was the efficacy of the commercial formulation consisting of gelatin capsules containing $NaHCO_3$ and lyophilized vaccine that was marketed after the Egyptian field trial?

5) Could prolongation of the interval between the doses enhance the immunogenicity of the vaccine?

6) Could an immunologic assay be identified that would correlate with levels of vaccine efficacy in field trials and could therefore be used to predict the effect of changes in formulation and immunization schedules?

In order to answer these questions, four separate field trials of efficacy were carried out in Santiago, Chile. These trials represent a collaborative effort involving the Ministry of Health, Santiago, Chile, the Center for Vaccine Development of the University of Maryland School of Medicine, the Pan American Health Organization, the World Health Organization, the Swiss Serum and Vaccine Institute, and the Walter Reed Army Institute of Research.

Field Trial Designs

The first two field trials were placebo-controlled and were initiated in the Northern (Area Norte) and Western (Area Occidente) administrative areas of Santiago in 1982 and 1983, respectively. The third field trial was begun in the Southern (Area Sur) and Central (Area Central) administrative areas of Santiago in 1984. Santiago, Chile was selected as the site for these field trials because of the combination of high endemicity of typhoid fever (the annual incidence rate from 1977 to 1981 exceeded 150 cases per 10^5 population) (31), the presence of a reknowned health care infrastructure (the National Health Service), a strong commitment on the part of the Ministry of Health towards innovative methods to control typhoid fever, and a long history of school-based

vaccination programs.

Only children of consenting parents entered the studies and were randomized to the various cells of the trials. Remaining children of non-consenting parents were also kept under surveillance and served as unvaccinated controls.

Since typhoid fever exhibits a marked seasonality (November to April) in conjunction with summer in Santiago (31), the vaccinations were limited to the cool months of the year (May to October). Computerized data files were generated from the completed class lists.

Only bacteriologically-confirmed cases (i.e. those from whom S. typhi was isolated from blood, bone marrow, or bile-stained duodenal fluid) were utilized in computations of vaccine efficacy. Therefore considerable resources were directed toward bacteriologic confirmation of suspect cases. Children admitted to hospital with a clinical suspicion of typhoid fever had three 4 ml blood cultures and one bone marrow culture obtained (32), while those presenting to the consultorios (health centers) as outpatients with suspect typhoid fever had two 6 ml. blood cultures drawn 30 minutes apart.

Chronologically, the Area Norte field trial preceeded the Area Occidente field trial. However, for purposes of clarity of presentation, the sequence of presentation of data will be Area Occidente, followed by Area Norte, and finally Area Sur and Central.

Area Occidente Field Trial

Parents of 96% of the 141,127 children in Area Occidente consented for their children to participate. These were thereupon randomized to one of five groups to receive:

Group 1 - Three doses of vaccine in enteric-coated capsules given with an interval of two days between the doses.

Group 2 - Three doses of vaccine with $NaHCO_3$ given with an interval of two days between the doses. The commercial gelatin capsule formulation was used which consisted of two gelatin capsules each containing 0.5 gm of $NaHCO_3$ and a third gelatin capsule containing lyophilized vaccine.

Group 3 - Three doses of vaccine in enteric-coated capsules with an interval of 21 days between the doses.

Group 4 - Three doses of the commercial gelatin capsule formulation with an interval of 21 days between the doses.

Group 5 - Three doses of placebo given with an interval of two days between the doses.

Mass administration of vaccine (containing 1-3 x 10^9 viable vaccine organisms per dose) or placebo was carried out between mid July and mid September, 1983 and surveillance began on September 21, 1983. In total, 109,594 children received all three scheduled doses of vaccine or placebo.

Results of three years of surveillance in the Area Occidente field trial are shown in Tables 4 and 5. The main points are:

1) The enteric-coated formulation was very significantly superior to the gelatin capsule/$NaHCO_3$ formulation (Table 4).

2) Increasing the interval between doses to 21 days offered no advantage to administering all three doses within one week (Table 4).

3) The level of protection (67% vaccine efficacy) conferred by the best regimen in the Occidente field trial (three doses of enteric-coated capsules given within one week) persisted for at least three years of surveillance (Table 5).

Surveillance is being maintained in Area Occidente to determine if the

efficacy of Ty21a can endure for more than three years. This information is critical for public health authorities to design typhoid control programs based on the systematic use of Ty21a.

Area Norte Field Trial

Parents of 92,356 of the 137,697 schoolchildren in Area Norte consented for their children to participate and they were randomized to one of three groups to receive:

1) Two doses of Ty21a vaccine in enteric-coated capsules ($1-3 \times 10^9$ organisms per dose).

2) One dose of vaccine and one dose of identical appearing placebo.

3) Two doses of placebo.

The two doses of vaccine or placebo were given to the children one week apart in May and June, 1982 and surveillance began on July 1, 1982.

Results of the Area Norte field trial are shown in Table 6. The main points include:

1) Two doses of enteric-coated vaccine provided moderate (48-72%) protection for a period of two years. However, the efficacy then dropped to 21% in the third season and was non-existent by the fourth season of surveillance.

2) A single dose of vaccine in enteric-coated capsules provided low levels of protection (16-39%) for two years but by the third year of surveillance no further efficacy was demonstrable.

These data demonstrate that, when administered in enteric-coated capsules, Ty21a provides insufficient levels of protection when given as only one or two doses.

Area Sur and Area Central Field Trials

A third field trial was undertaken in 1984 in Areas Sur and Central

where 247,561 children were randomized to receive either two, three or four doses of Ty21a vaccine ($1-3 \times 10^9$ viable vaccine organisms per dose) in enteric-coated capsules with all doses of vaccine being administered within a period of eight days in September and October, 1984. No placebo control group was included in this trial in which surveillance began on November 1, 1984.

Results of surveillance of typhoid fever through two seasons are shown in Table 7. In this trial the incidence of typhoid fever in recipients of three doses of Ty21a in enteric-coated capsules was only slightly lower than the incidence in children who received two doses of vaccine. In contrast, the incidence of typhoid fever in recipients of four doses of vaccine was very significantly lower than the rates in children who received two or three doses.

Area Sur Oriente Trial

In October, 1986, a fourth field trial was initiated in the Area Sur Oriente and Area Norte administrative areas where children received within one week three doses of Ty21a or placebo in either enteric-coated capsules or in a liquid formulation. Results of this trial (available in 1988) should answer the question of whether a liquid formulation of Ty21a, similar to what was used in Egypt, is inherently superior to enteric-coated capsules. This trial will also provide information on the absolute efficacy conferred by each formulation of vaccine.

A field trial similar in design to the above, using the identical liquid and enteric-coated capsule formulations of Ty21a, is concomitantly being carried out in Plaju, Indonesia, under the auspices of the Indonesian National Institute of Health and Ministry of Health with collaboration of the U.S. Naval Medical Research Unit, Djakarta, the World

Health Organization, and the Swiss Serum and Vaccine Institute.

Epidemiologic Evidence for a "Herd Immunity" Effect Consequent to the Broad Application of Ty21a Vaccine

Analysis of the incidence rate of typhoid fever in the placebo control group in the first field trial of Ty21a in Area Norte, Santiago provides some fascinating insights on what might be expected from the systematic wide-scale application of Ty21a live oral vaccine in typhoid fever control programs. As seen in Table 6, the incidence rate in the randomized control group in the first year of surveillance was 210 cases/10^5 schoolchildren. This rate of culture-confirmed cases is similar to the reported rate for schoolchildren in Area Norte in the period 1977-1981, prior to the field trial; however, at that time cases were not bacteriologically confirmed.

Surveillance of the second typhoid season in Area Norte took place after most of the children in adjacent Area Occidente had been given vaccine as part of the second field trial of Ty21a. The incidence rate in the placebo control group in Area Norte in this second year of surveillance fell to 141 cases/10^5 (Table 6).

Shortly before the third typhoid season of surveillance began in Area Norte, more than 247,000 children in Areas Sur and Central were given two, three or four doses of vaccine. In this third year of surveillance the incidence in the placebo group in Area Norte fell even further to 69 cases/10^5 (Table 6). A rate this low had not been encountered in Area Norte for decades.

The fourth year of surveillance in the Area Norte field area occurred during a year when no further trials were carried out in Santiago. Notably, in that fourth year the incidence of typhoid fever in the placebo

control group did not fall further. Rather, the incidence, 78 cases/10^5, closely resembled that of the previous year (Table 6).

In the course of the first three field trials in Santiago, approximately 65% of the schoolchildren in the city have participated, many having received an efficacious formulation and number of doses of vaccine. Thus, one interpretation of the sharp decrease in incidence rates in the placebo control group in the Area Norte trial is that this is the consequence of the mass application of Ty21a vaccine in schoolchildren.

Correlation of IgG ELISA S. typhi O Antibody with Efficacy in Field Trials

Serologic studies have been carried out in healthy Chileans, age 17-21 years, who received Ty21a in one of two formulations and in various immunization schedules. Serum IgG and IgA antibodies to S. typhi O antigen have been measured before and after vaccination by an ELISA that has been described in detail (32). Now that results of the field trials are available, it has become possible to relate seroconversion rates to vaccine efficacy; these comparisons are summarized in Table 8. It is obvious that there exists a positive correlation between the seroconversion rate of IgG S. typhi O antibody and vaccine efficacy in the field.

Ty21a Vaccine in Perspective

The great advantage of Ty21a live oral typhoid vaccine, in comparison with parenteral killed whole cell vaccines, is that it provides significant protection without causing adverse reactions (34). A wealth of evidence from volunteer studies (28) and from some of the largest vaccine field trials ever carried out attest to the biological activity of

this attenuated strain in providing protection against typhoid fever. Considerable resources have been expended in attempts to identify an effective and practical formulation and dosage schedule for Ty21a. After a series of field trials in Egypt and Chile, information has now been accrued demonstrating both the advantages as well as the limitations of Ty21a.

Field trials in Chile have shown that Ty21a in enteric-coated capsules is significantly more protective than vaccine administered in the gelatin capsule/$NaHCO_3$ formulation. These results corroborate a retrospective study reporting poor efficacy for the gelatin capsule/$NaHCO_3$ formulation (35) which prior to the Chilean trial had not been field tested. Following results of the Chilean field trials, production of the gelatin capsule/$NaHCO_3$ formulation was discontinued and replaced commercially by the enteric-coated capsule formulation.

In the Chilean trials, three doses of an enteric-coated formulation of Ty21a given within one week provided 67% protection for at least three years (Table 5). This level of vaccine efficacy is equal to the protection conferred by the highly reactogenic liquid heat-phenol-inactivated parenteral vaccine, the only other widely available effective vaccine (16,17,19, Table 1). The phenol-inactivated vaccine, which causes notable adverse reactions in approximately 25% of recipients, must be administered by needle and syringe or jet gun. Thus, Ty21a is distinctly more advantageous because it causes no discernible adverse reactions and is easy to administer to schoolchildren in mass vaccinations (34). In our estimation, this clearly makes Ty21a at present the vaccine of choice for any country intending to embark on a systematic typhoid fever control program.

The 67% protection conferred for at least three years by three doses of enteric-coated capsules given within one week in Area Occidente in Santiago, Chile is less than the impressive 96% efficacy over three years provided by a liquid formulation in Alexandria, Egypt. Besides the obvious differences in vaccine formulation and genetic constitution of the populations, other factors may have contributed to the difference in results. For example, the mean annual incidence rate in the placebo control group in the Occidente trial ($103/10^5$/year) was twice as high as the mean annual incidence rate in the placebo group in the Alexandria trial ($46/10^5$), suggesting that force of infection and modes of transmission may differ between the two sites. A fourth field trial currently underway in Chile, and a trial of similar design in Indonesia, will directly answer the question of the relative efficacy of enteric coated capsules versus a liquid formulation.

Widespread vaccination with Ty21a apparently created a "herd immunity" effect in which the incidence increasingly dropped in the control group in the first field trial area as children in other areas of the city were vaccinated. These observations support the contention that Ty21a live oral vaccine, while not the ideal anti-typhoid vaccine, is nevertheless a credible weapon to be employed in systematic typhoid fever control programs. Since man is the only reservoir, as well as the only natural host, of this infection, this approach is epidemiologically rational.

The multiple field trials of efficacy of Ty21a that have been required so far to generate the information necessary to determine how to use this vaccine as a public health tool are reminiscent of the series of field trials undertaken by WHO over a period of more than 15 years to accrue similar information for the parenteral killed whole cell vaccines. Until

a superior formulation of Ty21a is identified, or Ty21a is surpassed by another typhoid vaccine, the information now available should allow public health authorities to utilize Ty21a in enteric-coated capsule formulation as a tool in national typhoid fever control programs.

DEVELOPMENT OF AUXOTROPHIC ARO-, PUR- MUTANTS OF S. TYPHI AS LIVE ORAL VACCINES

Vaccine strain 541Ty was derived by Stocker and coworkers (36) from a wild strain of S. typhi by transducing deletions in two separate genes, each previously characterized in S. typhimurium and affecting a different pathway such that the mutations cause requirements for metabolites that are unavailable in mammalian tissues and intercellular fluid. The deletion mutation of gene aroA creates a requirement for several aromatic compounds, including p-aminobenzoic acid and 2,4-dihydroxybezoic acid, which are not mammalian metabolites. The second deletion mutation, at gene purA, causes a specific requirement for adenine (or an assimilable compound such as adenosine) (37). These nutritional requirements render strain 541Ty unable to sustain growth in mammalian tissues. Strain 543Ty is a derivative of 541Ty that lacks the Vi antigen.

Strain 541Ty or 543Ty were administered orally with $NaHCO_3$ to 33 healthy young adult volunteers in single doses of 10^8, 10^9, or 10^{10} organisms, while four additional vaccinees ingested two 2×10^9 organism doses four days apart, in preliminary evalutions of the safety and immunogenicity of the vaccine strains (33). No notable adverse reactions such as fever, diarrhea, vomiting, or abdominal discomfort were observed during 15 days of surveillance in a Research Ward or for two weeks thereafter. Vaccine organisms were recovered from coprocultures of 29 of 37 vaccinees (78%) and from duodenal cultures of two individuals; in

contrast, repeated blood cultures were negative. The humoral antibody response to S. typhi O and H antigens in serum and intestinal fluid was meager; no vaccinees had rises in serum antibody to S. typhi Vi or lysate antigen. However, all vaccinees manifested cell-mediated immune responses. After vaccination, 72% of recipients of doses of $\geq 10^9$ vaccine oragnisms responded to S. typhi particulate or purified O polysaccharide antigens in lymphocyte replication studies but not to antigens of other Salmonella or Escherichia coli. All individuals, after vaccination, demonstrated a significant plasma-dependent mononuclear cell inhibition of wild S. typhi. These preliminary results suggest that Aro-auxotrophic mutants of S. typhi are safe and immunogenic oral vaccines in man and are worthy of expanded clinical trials. The possible advantage of strain 541Ty, should it prove to be protective, is that its method of preparation involves the creation of precise deletion mutations in specific genes that do not otherwise affect the antigenic make-up of the S. typhi.

ATTENUATED S. TYPHI VACCINE STRAINS EXPRESSING GENES OF OTHER ORGANISMS

Because they are so well-tolerated and stimulate both humoral and cell-mediated immune responses (33, 38-41), attenuated S. typhi oral vaccines are attractive as carriers of critical genes of other organisms. The expression of foreign genes in strains such as Ty21a results in bivalent vaccines. Ty21a, for example, has been modified to express Shigella sonnei O antigen (42), the B subunit of E. coli heat-labile enterotoxin (43), colonization factor antigen I (44), and Vibrio cholerae antigens (45). In each of these instances Ty21a contains a plasmid encoding the relevant antigen of another enteropathogen. The bivalent typhi/S. sonnei vaccine has undergone extensive clinical testing in humans

and is safe, immunogenic and protective (although lot-to-lot variation has been described) (46,47). Ty21a expressing V. cholerae Inaba O antigen has been found to be well-tolerated and to stimulate circulating and local intestinal antibodies to both typhi and V. cholerae O antigens (48).

DEVELOPMENT OF HIGHLY PURIFIED VI POLYSACCHARIDE AS A PARENTERAL TYPHOID VACCINE

The Vi polysaccharide of S. typhi is a homopolymer of alpha-1,4 2-deoxy-2-N-acetyl galacturonic acid that covers the bacteria as a capsular antigen and is a known virulence property. The Vi antibody response following acute illness is usually modest, detectable in only a minority of patients, and short-lived, except in chronic biliary carriers of S. typhi who maintain very elevated levels of Vi antibody (49,50). Historically, several investigators have proposed that protection against typhoid fever may be feasible if high titers of Vi antibody can be elicited. It is known, however, that highly significant protection against S. typhi can be exhibited in the absence of Vi antibody, since Ty21a lacks Vi antigen and does not stimulate Vi antibody.

In the early 1950s, Landy (51) prepared purified Vi polysaccharide from acetone-inactivated bacteria by multiple extractions with saline, ethanol, and acetic acid. This early method may have partially denatured the antigen, resulting in a loss of O-acetyl and N-acetyl moieties (52,53). Landy's Vi preparation was immunogenic (51,54) but did not provide significant protection to volunteers in a small experimental challenge study carried out by Hornick et al (55) in the 1960s.

In attempts to purify Vi under non-denaturing conditions, Wong et al (56) and Robbins and Robbins (53), treated S. typhi with hexadecyltrimethylammonium bromide, a detergent that was previously

instrumental in the preparation of purified meningococcal polysaccharide vaccines (57). Two separate lots of Vi vaccine prepared by this procedure, one made at the National Institutes of Health in the U.S.A. (Lot 53226) and the other made at the Merieux Institute, Lyon, France (Lot IMS 1569) were evaluated for safety and immunogenicity (58). The NIH vaccine contained approximately 5% residual lipolysaccharide (LPS), while the French vaccine had only 0.2% LPS. Both vaccines elicited significant rises in Vi antibody in circa 90% of recipients but the NIH preparation caused some systemic and local adverse reactions. The occurrence of significant rises in O antibody in 83% of recipients of the NIH vaccine, suggest that residual LPS was responsible for the untoward reactions.

Vi vaccine prepared by the Merieux Institute is currently being evaluated in controlled field trials of efficacy in Nepal and South Africa. Preliminary results from these trials should be available in 1987 (personal communications, J.B. Robbins and H. Koornhoff).

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Table 1. Results of controlled field trials of lyophilized acetone-inactivated and heat phenol-inactivated reference vaccines

Field Site, Dates	Age Groups	Vaccine (No. Doses)	No. Vaccinated	Duration of Surveillance	Incidence of typhoid per 10^5	Vaccine Efficacy	Reference
Yugoslavia, 1960-63	2-50 yrs. Mostly Schoolchildren	K (2)	5028	2 1/2 yrs.	318[a]	79%	16
		L (2)	5068	2 1/2 yrs.	727[b]	51%	
		Control (2)	5039	2 1/2 yrs.	1488[c]	---	
Guyana, 1960-67	5-15 yrs. (Schoolchildren)	K (2)	24,046	7 yrs.	71[d]	88%	17
		L (2)	24,241	7 yrs.	198[e]	67%	
		Control (2)	27,756	7 yrs.	605[f]	---	
Poland, 1961-64	5-14 yrs. (Schoolchildren)	K (2)	81,534	3 yrs.	7[g]	88%	18
		Control (2)	83,734	3 yrs.	47[h]	---	
USSR, 1962-65	Schoolchildren and young adults (92% age 7-15 yrs.)	L (2)	36,112	2 1/2 yrs.	55[i]	66%	19
		Control (2)	36,999	2 1/2 yrs.	162[j]	---	

a vs c, $p = 0.00001$ a vs c, $p = 0.0064$ e vs f, $p < 0.000005$ g vs h, $p = 0.0000025$
b vs c, $p < 0.0004$ d vs f, $p < 0.000001$ d vs e, $p = 0.000046$ i vs j, $p = 0.000021$, all comparisons by Chi square.

Table 2. The frequency of fever, malaise and pain at the injection site approximately 24 hours following subcutaneous inoculation with heat-phenol-inactivated (vaccine L) or acetone-inactivated (vaccine K) whole cell typhoid vaccines or tetanus toxoid

Vaccine	No. of Vaccinees			Fever after Vaccination (%)			Inability to Work (%)	Local Pain (%)	
Group	Yugoslavia	Guyana	USSR	Yugoslavia*	Guyana+	USSR++	Yugoslavia	Yugoslavia	Guyana
Heat-phenol-inactivated	345	86	1656	24	29	6.7	23	35	54
Acetone-inactivated	326	80	-	22	26	-	21	32	45
Tetanus toxoid	328	86	1757	3	7	2.4	5	4	

* > 37°C
\+ > 37.8°C
++ > 37.5°C

Data summarized from references 16, 19 and 21

Table 3. Field trial of efficacy of three doses of a liquid formulation of Ty21a vaccine given with $NaHCO_3$ to six and seven year old schoolchildren in Alexandria, Egypt. Results of three years of surveillance.

Year of observation	Confirmed cases of typhoid fever	Annual incidence per 10^5	Vaccine efficacy (%)
1978-1979			
vaccinees*	0	0	100
placebo+	7	44	
1979-1980			
vaccinees	0	0	100
placebo	8	50	
1980-1981			
vaccinees	1	6	86
placebo	7	44	
Total 1978-1981			
vaccinees	1	-	96
placebo	22	-	

Data from reference 29
* n = 16, 486
\+ n = 15, 502

Table 4. Comparison of the efficacy of two different formulations of Ty21a vaccine administered in two different immunization schedules in Area Occidente, Santiago, Chile. Results of 36 months of surveillance, 9/83 - 8/86

	Enteric-Coated Capsules		Gelatin Capsules with $NaHCO_3$		
	Long Interval* (21,598)	Short Interval+ (22,170)	Long Interval (21,541)	Short Interval (22,379)	Placebo (21,906)
Cases	34	23	46	56	68
Incidence/10^5	157.4[a]	103.7[b]	213.5[c]	250.3[d]	310.4[e]
Efficacy	49.3	66.6	31.2	19.3	---

* 3 doses, 21 days between doses
\+ 3 doses, 1-2 days between doses

a vs e, p = 0.0006
b vs e, p < 0.00001
c vs e, p = 0.023
d vs e, p = 0.21
a vs c, p = 0.23
b vs d, p = 0.00052
a + b vs c + d, p = 0.001
All statistical comparisons by Chi square

Table 5. Duration of the efficacy conferred by three doses of the enteric-coated capsule formulation of Ty21a live oral vaccine given within one week in Area Occidente, Santiago, Chile

	Vaccine* (22,170)	Placebo* (21,906)
Year 1 (9/83-8/84)		
Cases	7	24
Incidence/10^5	31.6	109.6
Efficacy	71.2	-
Year 2 (9/84-8/85)		
Cases	8	20
Incidence/10^5	36.1	91.3
Efficacy	60.5	-
Year 3 (9/85-8/86)		
Cases	8	24
Incidence/10^5	36.1	109.6
Efficacy	67.1	-
Total Years 1-3 9/83-8/86		
Cases	23	68
Incidence	103.7[a]	310.4[b]
Efficacy	66.5	-

* 3 doses, 1-2 days between doses

a vs b, $p < 0.00001$, Chi square

Table 6. Comparison of the efficacy of one versus two doses of Ty21a live oral typhoid vaccine given in enteric-coated capsule formulation. Randomized, controlled, double-blind trial in Area Norte, Santiago, Chile

	One Dose (32,788)	Two Doses (27,620)	Placebo (31,948)
Year 1 (7/82-6/83)			
Cases	58	30	67
Incidence/10^5	176.9[a]	108.6[b]	209.7[c]
Efficacy	15.6%	48.2%	-
Year 2 (7/83-6/84)			
Cases	28	11	45
Incidence/10^5	85.4	39.8	140.8
Efficacy	39.3%	71.7%	-
Year 3 (7/84-6/85)			
Cases	23	15	22
Incidence/10^5	70.1	54.3	68.9
Efficacy	0%	21.2%	-
Year 4 (7/85-6/86)			
Cases	33	22	25
Incidence/10^5	100.6	79.6	78.3
Efficacy	0%	0%	-

a vs c, p = 0.42
a vs b, p = 0.037
b vs c, p = 0.0032
Comparisons by Chi square

Table 7. Comparison of the efficacy of two, three, and four doses of Ty21a vaccine in enteric-coated capsule formulation. Results of a randomized field trial in Area Sur and Area Central, Santiago, Chile.

Surveillance from 10/84 to 9/86	Two Doses*	Three Doses*	Four Doses*
No. of Vaccinees	93,942	95,198	58,421
Cases	126	117	34
Incidence/10^5	134.1[a]	122.9[b]	58.2[c]

* Vaccine given within eight days with 1-2 days between doses

a vs c, $p < 0.0001$
b vs c, $p < 0.0002$
a vs b, $p = 0.49$
All comparisons by Chi square

Table 8. Rates of seroconversion of IgG-ELISA S. TYPHI O antibody following one to three oral doses of Ty21a live oral typhoid vaccine given within one week. Comparison of two different formulations.

Formulation	No. Doses	Seroconversion Rate (%)	Vaccine Efficacy in Controlled Field Trials*
Enteric-coated capsules	3	61/96 (64)	67%
	2	22/50 (44)	47%
	1	9/50 (18)	21%
Vaccine + $NaHCO_3$ in gelatin capsules	3	99/195 (50)	19%

* Data from first 36 months of surveillance in field trials in Area Norte and Area Occidente, Santiago, Chile.

LARGE-SCALE FIELD TRIAL OF TY21A LIVE ORAL TYPHOID VACCINE IN ENTERIC-COATED CAPSULE FORMULATION

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ABSTRACT

Three doses, given within one week, of Ty21a attenuated Salmonella typhi oral vaccine in an enteric-coated capsule formulation provided 67% efficacy for at least three years in a randomized, placebo-controlled field trial involving 109,000 schoolchildren in Santiago, Chile. Increasing the interval between doses to 21 days did not enhance protection. Significantly less protection followed administration of vaccine in gelatin capsules with $NaHCO_3$. Ty21a provides the same level of protection as the heat-phenol-inactivated whole cell parenteral vaccine but in contrast does not cause adverse reactions. Ty21a may now be considered a practical public health tool.

INTRODUCTION

Typhoid fever remains an important public health problem in many less-developed regions of the world and poses a risk for travelers.[1-4] In most endemic areas the incidence of typhoid fever is highest in children 5-19 years of age.[5-9] This is of potential relevance in the control of typhoid, since schoolchildren represent a "captive" population amenable to school-based immunization programs.

Although heat-phenol-inactivated and acetone-inactivated parenteral killed whole cell typhoid vaccines confer significant protection,[10-13] they are rarely used by any country in systematic typhoid fever control programs because of the high rates of adverse reaction that they elicit.[10,13,14]

An important advance in immunization against typhoid fever was the development by Germanier and Furer[15] of an attenuated strain of S. typhi, Ty21a, that can be utilized as a live oral vaccine. In preliminary studies in adult volunteers in North America, Ty21a caused no adverse reactions, was genetically stable, and significantly protected against experimental infection.[16]

Wahdan et al[17,18] carried out a placebo-controlled, randomized, double-blind trial of Ty21a in Alexandria, Egypt where three doses of vaccine ($1-3 \times 10^9$ viable vaccine organisms per dose) or placebo were given to 32,000 schoolchildren on Monday, Wednesday, and Friday of one week. Prior to ingestion of liquid (reconstituted lyophilate) vaccine or placebo, each child chewed a 1.0 gm $NaHCO_3$ tablet to neutralize gastric acid. Notable adverse reactions were not detected, corroborating the safety of the live oral vaccine. During 36 months of surveillance the vaccine efficacy was 96%[18].

Shortly after the Egyptian field trial, the Swiss Serum and Vaccine Institute made available commercially a formulation of vaccine consisting of two gelatin capsules each containing $NaHCO_3$ and a third gelatin capsule containing lyophilized vaccine. This first commercial formulation resembled, but was not identical to, that used in Alexandria.

Despite the highly encouraging results in the field trial in Egypt, additional information had to be obtained before Ty21a could be employed as a practical public health tool. Several critical questions had yet to be answered. What would be the efficacy of the commercial gelatin capsule/$NaHCO_3$ formulation of lyophilized vaccine that was marketed after the Egyptian field trial? What would be the efficacy of Ty21a in a formulation, such as enteric-coated capsules, that does not require $NaHCO_3$? What level of protection would Ty21a provide in areas with incidence rates of typhoid fever much higher than the 44-50 cases/10^5/year that prevailed during the trial in Alexandria? Could prolongation of the interval between the doses enhance the immunogenicity of the vaccine?

To answer these questions, a randomized, placebo-controlled field trial of efficacy was carried out in Santiago, Chile. This trial represented a collaborative effort involving the Ministry of Health, Santiago, Chile, the Center for Vaccine Development of the University of Maryland School of Medicine, the Pan American Health Organization, the World Health Organization (WHO), the Walter Reed Army Institute of Research and the Swiss Serum and Vaccine Institute.

MATERIALS AND METHODS

Santiago was selected for the field trial because of the high endemicity of typhoid fever (the annual incidence rate from 1977 to 1981

exceeded 150 cases per 10^5 population),[19] the presence of an excellent health care infrastructure (the System of National Health Services), a strong commitment on the part of the Ministry of Health towards innovative methods to control typhoid fever, and a long history of school-based vaccination programs.[20] The trial design and consent procedures were approved by ethical review committees of the University of Maryland and WHO. The field trial was initiated in Area Occidente of Santiago in 1983. The Ministries of Health and Education collaborated to ensure that, with cooperation of the teachers in all schools, parents were informed of the trial (by means of health education brochures) and permission to enroll their child was requested through consent forms and their response recorded.

Peak transmission of typhoid fever and the vast majority of cases occur during the summer (school holiday) season in Santiago (mid-December to mid-March) while schools are not in session.[19] Therefore, randomization was carried out by classroom (i.e. all children in a class received the same vaccine regimen).

Only children of consenting parents were randomized to one of the five cells of the trial. Group 1 received three doses of vaccine with $NaHCO_3$ given with an interval of two days between the doses. The commercial gelatin capsule formulation was used which consisted of two gelatin capsules each containing 0.5 gm of $NaHCO_3$ and a third gelatin capsule containing lyophilized vaccine. Group 2 ingested three doses of lyophilized vaccine in enteric-coated capsules, with an interval of two days between the doses. Hydroxyprophy-methyl-cellulose-phthalate was the enteric-coating used to make the gelatin capsules acid-resistant. In vitro the capsules resisted gastric acid (pH 1.5) for at least two hours

but dissolved within 10 minutes in artificial intestinal fluid of pH $\geq$6.0. Group 3 received three doses of vaccine in enteric-coated capsules with an interval of 21 days between the doses. Group 4 ingested three doses of the commercial gelatin capsule/$NaHCO_3$ formulation with an interval of 21 days between the doses, while Group 5 received three doses of placebo (in identical capsules as described above) given at an interval of two days between the doses. The identity of which coded preparation contained placebo was unknown to the vaccinators, the schoolchildren and the health care providers.

The administration of vaccine (containing 1-3 x 10^9 viable vaccine organisms per dose) or placebo by trained health workers was carried out in the classrooms in the cool, non-typhoid season, mid-July to early September, 1983; surveillance began on September 21, 1983. Computerized data files were generated from the completed class lists.

Approximately 90% of health care visits in Area Occidente occur in facilities of the System of National Health Services where intensive surveillance could be maintained; the remaining visits involve private physicians. Physicians and nurses were kept aware of the importance of obtaining cultures from suspect cases of typhoid fever by means of letters, clinical conferences and weekly visits by surveillance nurses from the Ministry of Health. Only cases confirmed bacteriologically (i.e. those from whom S. typhi was isolated from blood, bone marrow, or bile-stained duodenal fluid) were utilized in computations of vaccine efficacy. Therefore, considerable resources were directed toward bacteriologic confirmation of suspect cases. Three 4 ml blood cultures and one bone marrow culture[21] were obtained from children admitted to hospital with a clinical suspicion of typhoid fever. Two 6 ml. blood

cultures, drawn 30 minutes apart, were collected from outpatients presenting to the consultorios (health centers) with suspected typhoid fever. Suspicious colonies were confirmed by standard biochemical and serological techniques.[22]

The code for this blinded study was kept in Berne and Geneva. After breaking the code, the results were analyzed by Chi square.

RESULTS

Parents of 96% of the 141,127 children in Area Occidente gave consent for their children's participation. In total, 109,594 schoolchildren 6-21 years of age (99% were 6-19 years old) received all three scheduled doses of vaccine or placebo. During the vaccination period in the schools there was no increased absenteeism or notable increase in febrile or intestinal illnesses and no cases of typhoid fever were recorded among the participating children.

Results of three years of surveillance in the Area Occidente field trial are summarized in Table 1 where incidence is presented both as cases per 10^5 schoolchildren as well as by classes with cases per 100 classes vaccinated (since randomization was done by class). The 227 confirmed cases of typhoid occurred in 221 separate classes. For those few classes with more than one case, they occurred in different years of surveillance; thus there were no clusters of cases. Vaccine efficacy was virtually identical whether calculated on the basis of incidence per 10^5 schoolchildren or classes with typhoid per 100 classes. The enteric-coated capsule formulation gave significantly better protection than the gelatin capsule/$NaHCO_3$ formulation. The best protection occurred in the group which received vaccine in enteric-coated capsules with all three doses given within one week (as in the Egyptian trial);

prolonging the interval between doses to 21 days did not enhance efficacy.

For the regimen (enteric-coated capsules, short interval) with the best protection (67% efficacy), the efficacy during each year of surveillance is presented in Table 2 and shows that the level of protection remained similar for all three years (61-71%). Surveillance is being maintained in Area Occidente to determine whether protection endures beyond three years. With this regimen fewer cases of enteric fever due to S. paratyphi B (10 cases, 45.1 cases/10^5) were observed than in the placebo group (17 cases, 77.6 cases/10^5; 45% vaccine efficacy) but the difference was not significant ($p=0.24$).

The relationship between age at vaccination and level of efficacy is shown in Table 3 for the group who received three doses of enteric-coated vaccine given within one week. While significant protection occurred in all age groups, there was a clear-cut trend suggesting that the level of protection increased with age at the time of vaccination; however, the differences in efficacy were not statistically significant.

DISCUSSION

The great advantage of Ty21a live oral typhoid vaccine, compared to parenteral killed whole cell vaccines, is that it provides significant protection without causing adverse reactions,[16,17,23-25] A wealth of evidence from volunteer studies[16] and from large vaccine field trials attests to the protective activity of this attenuated strain. The field trial from Chile, reported herein, evaluating different formulations and immunization schedules, provides practical information on both the advantages and the limitations of Ty21a as a possible control measure against typhoid fever in endemic areas.

The field trial in Area Occidente has shown that Ty21a in

enteric-coated capsules is significantly more protective than vaccine in the gelatin capsule/$NaHCO_3$ formulation (Table 1). These results corroborate a retrospective study reporting poor efficacy for the gelatin capsule/$NaHCO_3$ formulation,[26] which prior to the Chilean trial had not previously been field tested. Based on the Area Occidente results, production and marketing of the gelatin capsule/$NaHCO_3$ formulation was discontinued and replaced by the enteric-coated capsule formulation.

In Area Occidente, three doses of enteric-coated Ty21a given within one week provided 67% protection for at least three years (Table 2). This level of vaccine efficacy is equal to the protection conferred by the heat-phenol-inactivated parenteral vaccine (Table 4), the only other widely available effective typhoid vaccine.[27] The phenol-inactivated vaccine, however, causes notable adverse reactions in approximately 25% of recipients and must be administered by needle and syringe or jet gun.[10,13,14] Thus Ty21a is distinctly more advantageous because it causes no discernible adverse reactions and is easy to administer to schoolchildren in mass oral vaccinations.[23,25] In our estimation, this clearly makes Ty21a at present the vaccine of choice for any country intending to embark upon a systematic typhoid fever control program.

The 67% protection conferred for at least three years by three doses of Ty21a in enteric-coated capsules given within one week in Area Occidence is less than the impressive 96% efficacy over three years provided by a liquid formulation used in Egypt (Table 4). Besides the obvious differences in vaccine formulation and genetic constitution of the populations, other factors may have contributed to the difference in results. For example, the mean annual incidence in the placebo group in the Occidente trial ($103/10^5$/year) was twice as high as the mean annual

incidence in the placebo group in the Alexandria trial ($46/10^5$), suggesting that force of infection and modes of transmission may differ between the two sites. Another WHO-sponsored field trial currently underway in Area Sur Oriente of Santiago and a similar trial in Indonesia, are directly comparing the relative efficacy of enteric coated capsules versus a liquid formulation.

Ty21a may also prove useful in the future in immunization against other infections. Because Ty21a stimulates cell-mediated as well as humoral immunity,[24,25,28-30] it is attractive as a carrier of relevant genes from other organisms. Ty21a, for example, has been modified to express Shigella sonnei O antigen,[31,32] the B subunit of E. coli heat-labile enterotoxin,[33] colonization factor antigen I,[34] and Vibrio cholerae antigens.

The multiple field trials that have been required to generate the information on how to use Ty21a as a public health tool are reminiscent of the series of field trials undertaken by WHO over more than 15 years to accrue similar information for the parenteral killed whole cell vaccines.[10-13] Until a superior formulation of Ty21a is identified, or it is surpassed by another typhoid vaccine, the information now available should allow public health authorities to utilize Ty21a in enteric-coated capsules in national typhoid fever control programs.

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Table 1. Comparison of the efficacy of two different formulations of Ty21a vaccine administered in two different immunization schedules in Area Occidente, Santiago, Chile. Results of 36 months of surveillance, 9/83 - 8/86

	Enteric-Coated Capsules		Gelatin Capsules with $NaHCO_3$		
	Long Interval*	Short Interval+	Long Interval	Short Interval	Placebo
No. children	21,598	22,170	21,541	22,379	21,906
No. classes	861	863	864	862	862
Cases	34	23	46	56	68
Incidence/10^5	157.4[a]	103.7[b]	213.5[c]	250.3[d]	310.4[e]
Efficacy	49% (24-66%)**	67% (47-79%)	31% (0-52%)	19% (0-43%)	---
Classes with typhoid	34	23	46	54	64
Classes with typhoid/100 classes	3.95[f]	2.67[g]	5.32[h]	6.26[i]	7.42[j]
Efficacy	47%	64%	28%	16%	---

* 3 doses, 21 days between doses
\+ 3 doses, 1-2 days between doses
** (95% confidence intervals of vaccine efficacy)

a vs e, p = 0.0006
b vs e, p < 0.00001
c vs e, p = 0.023
d vs e, p = 0.21
a vs c, p = 0.23
b vs d, p = 0.00052
a + b vs c + d, p = 0.001
f vs j, p = 0.00135
g vs j, p = 0.0000031
h vs j, p = 0.07
i vs j, p = 0.35
g vs i, p = 0.00032
f+g vs h+i, p = 0.00024

All statistical comparisons by Chi square

Table 3. Duration of the efficacy conferred by three doses of the enteric-coated capsule formulation of Ty21a live oral vaccine given within one week in Area Occidente, Santiago, Chile

	Vaccine* [22,170]	Placebo* [21,900]
Year 1 (9/83-8/84)		
Cases	7	24
Incidence/10^5	31.6	109.6
Efficacy	71% (36-87%)**	- -
Year 2 (9/84-8/85)		
Cases	8	20
Incidence/10^5	36.1	91.3
Efficacy	61% (12-82%)	- -
Year 3 (9/85-8/86)		
Cases	8	24
Incidence/10^5	36.1	109.6
Efficacy	67% (28-85%)	- -
Total Years 1-3 (9/83-8/86)		
Cases	23	68
Incidence	103.7[a]	310.4[b]
Efficacy	67% (47-79%)	- -

* 3 doses, 1-2 days between doses

** (95% confidence intervals of vaccine efficacy)

a vs b, $p < 0.00001$, Chi square

Table 3. Efficacy of three doses of Ty21a in enteric-coated capsules given within one week in relation to age of vaccinated children

	Placebo	Vaccinees	p-value
Age Groups			
5-9 years			
No. children	7193	7034	
No. cases	25	10	
Incidence/10^5 *	347.6	142.2	0.021
Efficacy	-	59% (16-80%) **	
10-14 years			
No. children	9710	9992	
No. cases	32	11	
Incidence/10^5	329.8	110.1	0.0018
Efficacy	-	67% (35-83%)	
≥ 15 years			
No. children	5001	5142	
No. cases	13	2	
Incidence/10^5	259.9	38.9	0.0082
Efficacy	-	85% (42-96%)	

* Results of 36 months of surveillance
** (95% confidence intervals of vaccine efficacy)

Table 4. Comparison of results of WHO-sponsored controlled field trials of lyophilized heat-phenol-inactivated parenteral killed whole cell typhoid vaccine (L) with results of field trials of attenuated S. typhi Ty21a live oral vaccine

Field Site, Dates	Age Groups	Vaccine (No. Doses)	No. Vaccinated	Duration of Surveillance	Incidence of typhoid per 10^5	Vaccine Efficacy	Reference
Yugoslavia, 1960-63	2-50 yrs. Mostly Schoolchildren	L (2)	5028	2 1/2 yrs.	727[a]	51%	10
		Control (2)	5039	2 1/2 yrs.	1488[b]	---	
Guyana, 1960-67	5-15 yrs. (Schoolchildren)	L (2)	24,241	7 yrs.	198[c]	67%	11
		Control (2)	27,756	7 yrs.	605[d]	---	
USSR, 1962-65	Schoolchildren and young adults (92% age 7-15 yrs.)	L (2)	36,112	2 1/2 yrs.	55[e]	66%	13
		Control (2)	36,999	2 1/2 yrs.	162[f]	---	
Egypt, 1978-81	Schoolchildren 6-7 yrs.	Ty21a (3)*	16,486	3 yrs.	6[g]	96% (77-99%)**	
		Control (3)	15,902	3 yrs.	133[h]		
Chile, 1983-86	Schoolchildren 6-21 yrs.	Ty21a (3)+	22,170	3 yrs.	104[i]	67% (47-79%)**	
		Control (3)	21,906	3 yrs.	310[j]		

* Liquid formulation, three doses given within one week
\+ Enteric-coated capsule formulation, three doses given within one week
** (95% confidence interval of vaccine efficacy)

a vs b, $p < 0.0004$ c vs d, $p < 0.000005$ e vs f, $p = 0.000021$ b vs e, $p < 0.000021$, i vs j, $p < 0.00001$, all comparisons by Chi square.

APPENDIX Q

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Safety and Immunogenicity of Two *Salmonella typhi* Vi Capsular Polysaccharide Vaccines

Typhoid fever remains a public health problem in many developing areas of the world. An easily administered, well-tolerated vaccine that produces a long duration of immunity after a single dose would be an important advance in controlling this disease. Inactivated whole-cell typhoid vaccines are effective but cause high rates of adverse reactions and require two injections for maximum protection in younger children [1]. Live, attenuated *Salmonella typhi* vaccine strain Ty21a is orally administered, well tolerated, and provides 70% (Chile) to 95% (Egypt) protection; however, this vaccine is limited in usefulness because it requires several doses to achieve this level of effectiveness [2].

The Vi capsular polysaccharide (CPS) is a linear homopolymer of α-1,4 2-deoxy-2-N-acetyl galacturonic acid; it is variably O-acetylated at C3 [3]. Measuring serum antibodies to Vi provides a sensitive and specific screening test for identifying chronic, asymptomatic carriers of *S. typhi* [4]. Some evidence suggests that Vi may be a protective immunogen. In an experimental challenge, volunteers immunized with Vi CPS were not protected against challenge with up to 10^7 *S. typhi* organisms [5]. The Vi CPS used in this study, however, was prepared under conditions that altered its structure [6]. New purification techniques using detergents that do not alter the structure of CPS led to the development of meningococcal and pneumococcal CPS vaccines. Vi CPS prepared by a similar nondenaturing technique was evaluated in a small number of volunteers; the vaccine elicited higher antibody responses to Vi and less-adverse reactions than did the acetone-killed, whole-cell vaccine [7, 8]. In this report we describe further studies assessing the immunogenicity and side effects of two Vi preparations in volunteers from nonendemic areas (Maryland and France) and from a hyperendemic area (Chile).

Subjects and Methods

Preparation and characterization of Vi CPS. *S. typhi* strain Ty-2 was cultivated in modified Frantz medium until stationary growth was reached [9]. For lot 53226, the culture was heated to 60 C for 1 hr, and 1% hexadecyltrimethylammonium bromide (Cetavlon®; Eastman Chemicals, Rochester, NY) was added. The suspension was centrifuged at 10,000 *g* for 20–30 min, and Vi was extracted from the pellet [9]. Lot IMS1569 was similarly prepared, except that *S. typhi* was removed by centrifugation and Cetavlon added to the supernatant; the resultant suspension was collected by centrifugation [10]. Vi CPS for passive HA and RIA was prepared from *Citrobacter freundii* strain WR7011 (provided by Dr. Louis Baron, Walter Reed Army Institutes of Research, Washington, DC). The two lots of Vi polysaccharide differed only in lipopolysaccharide (LPS) content (5% in lot 53226 and 0.2% in lot IMS1569) and in the minimal dose that was pyrogenic in rabbits (0.05 μg for lot 53226 and 0.5 μg for lot IMS1569).

Volunteers. Healthy volunteers were recruited at the University of Maryland at Baltimore. Forty-eight students (21–32 years of age) received by random assignment either 50 μg of Vi lot 53226 or 50 μg of meningococcal polysaccharide groups A, C, Y, and W-135 combined vaccine (Squibb Connaught, Princeton, NJ) by jet-gun injection. Volunteers were interviewed about symptoms (mild, moderate, or severe) and examined 24 and 48 hr after injection in a double-blind fashion. Temperatures were recorded for volunteers who complained of feverishness in the first 24 hr and for all volunteers 48 hr after vaccination.

In May, during the low-incidence season for typhoid fever in Chile, 189 Air Force recruits (18–21 years of age) volunteered for our study. One hundred thirty-six were randomly assigned to receive 50 μg of Vi lot 53226 and 53 to receive 50 μg of the tetravalent meningococcal polysaccharide vaccine by jet-gun injection. Temperatures were recorded every 12 hr, and volunteers were interviewed about symptoms and their severity and were examined 24 and 48 hr after injection in a double-blind fashion.

In Tours, France, 19 healthy medical students (20–24 years of age) were injected by syringe with 50 μg of Vi lot IMS1569. Rectal temperatures were taken 6, 24, 48, and 72 hr later. Symptoms and local reactions were recorded by the volunteers for 72 hr after injection.

Serology. Blood specimens from the Maryland students and the Chilean recruits were obtained before and 21 days after vaccination; blood from French volunteers was obtained before and 28 days after vaccination.

Passive HA. Washed, glutaraldehyde-treated sheep erythrocytes were sensitized with 10 μg of purified Vi antigen from *C. freundii* ml [4]. After adsorbing overnight with fresh sheep erythrocytes, sera were diluted from 1:20 to 1:2,560 in 1% sensitized erythrocytes; as a control, sera were diluted in nonsensitized erythrocytes. Plates were incubated for 2 hr at room temperature and examined for

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Informed consent was obtained from all volunteers, and the guidelines for human experimentation of the U. S. Department of Health and Human Services were followed in the conduct of these clinical studies.

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HA. Appropriate positive and negative control sera were used.

RIA. Tyramine (30 mg/ml; Sigma, St. Louis) was added to 10 mg of Vi polysaccharide/ml, and the pH was adjusted to 4.9. The water-soluble carbodiimide EDAC (0.05 *M*; BioRad, Richmond, Calif) was added, and the pH was maintained at 4.9–5.1 for 3 hr. The reaction mixture was dialyzed and passed through G-100 Sephadex® (Pharmacia, Piscataway, NJ) equilibrated in water; the void volume was freeze-dried. The final product contained 1.1% tyramine. A burro antiserum to Vi was used as a reference, and antibodies to Vi were determined by a modified Farr assay [9]. The sensitivity of the assay was 0.05 μg of antibody/ml, and the variability was ~15%. Seroconversion was defined as an increase in specific antibody to Vi ≥0.15 μg/ml. (This increase is >3 SD from the mean difference of paired sera from controls. The control population consisted of 30 individuals who had had an experimental oral challenge with *Vibrio cholerae*.)

ELISA. O-specific antibodies were measured by ELISA using LPS from *S. typhi* and *Salmonella enteriditis*. Alternating wells of polystyrene microtiter plates were coated for 1 hr at 37 C with 10 μg of a commercial LPS/ml; the LPS had been prepared by the hot-water phenol method from *S. typhi* strain 0901 (Difco, Detroit). Pre- and postvaccination sera were examined simultaneously. Aliquots of sera diluted 1:100 in PBS, 0.05% Tween 20, and 1% heat-inactivated fetal calf serum were applied in triplicate to wells of the microtiter plates; then alkaline phosphatase–conjugated goat antibody to human IgG and *p*-nitrophenol phosphate substrate (Kirkegaard and Perry, Gaithersburg, Md) were added. A standard ELISA procedure was used for washing and developing color [11]. Net OD was defined as the average increase in the OD of sample wells compared with wells without antigen. Seroconversion was defined as an increase in net OD ≥0.15 at 405 nm. (This increase is >3 SD from the mean difference of paired sera from controls. The control population consisted of the same 30 individuals described above.)

Sera were also assayed by ELISA for O-specific antibody using antigen prepared from *S. enteriditis* bioserotype *enteriditis* (Difco) to assure that antibodies to *S. typhi* LPS were O specific and were not antibodies to Vi antigen that might have contaminated the LPS preparation. The LPS of *S. typhi* and *S. enteriditis* bioserotype *enteriditis* are serologically indistinguishable [12]; however, the latter organism lacks Vi antigen. Counterimmunoelectrophoresis using rabbit (Centers for Disease Control, Atlanta) and burro hyperimmune *S. typhi* antiserum did not detect Vi antigen (sensitivity, 1.0 μg of Vi/ml) in either of these two LPS preparations at 1 mg/ml.

Results

Clinical responses. Table 1 shows the frequency of reactions in recipients of Vi lot 53226 and meningococcal vaccine. This Vi preparation produced a higher incidence of local and systemic reactions (rated moderate to severe) than did meningococcal vaccine ($P = .005$ for local reactions; the difference was not significant for systemic reactions, Fisher's exact test). Two Maryland students who received Vi had temperatures >37.5 C during the 48-hr observation period; one of these had a temperature of 39.1 C with malaise and myalgias and required bed rest.

Table 1. Reactions to two *S. typhi* Vi vaccine candidates.

	University of Maryland volunteers		Chilean Air Force recruits		French volunteers
Reactions	Vi lot 53226 (n = 24)	Meningococcal vaccine (n = 24)	Vi lot 53226 (n = 136)	Meningococcal vaccine (n = 53)	Vi lot IMS1569 (n = 19)
Systemic					
Malaise	8	0	3	2	0
Fever*	8	0	3	2	0
Went to bed	4	0	3	2	0
Local					
Local pain	29†	0	10	2	–
Tenderness	29‡	4	19‡	6	–
Erythema (≥2 cm)	71‡	38	21§	17	–
Induration (≥1 cm)	0	8	0	4	–

NOTE. Data are percentage of volunteers with moderate-to-severe reactions.

* For Maryland volunteers, the presence of fever indicated subjective fever or temperature >37.5 C (taken orally) 48 hr after vaccination; for Chilean volunteers, fever indicated temperature >37.5 C (taken orally) when measured every 12 hr for 48 hr after vaccination; for French volunteers, fever indicated temperature >37.6 C (taken rectally) when measured at 6, 24, 48, and 72 hr after vaccination.

† $P = .005$ by Fisher's exact test comparing recipients of Vi with recipients of meningococcal vaccine.

‡ $P < .05$ by Fisher's exact test comparing recipients of Vi with recipients of meningococcal vaccine.

§ $P < .001$ by χ^2 test comparing recipients of Vi with recipients of meningococcal vaccine.

Table 2. Immune response to two *S. typhi* Vi vaccine candidates.

Vi	No. of volunteers	Titers of antibody to Vi by Passive HA GMT Pre	Passive HA GMT Post	Passive HA Seroconversions (%)*	RIA GMT Pre	RIA GMT Post	RIA Seroconversions (%)†	Titers of antibody to *S. typhi* LPS by ELISA GMT Pre	ELISA GMT Post	ELISA Seroconversions (%)‡
Lot 53226										
Maryland students	24	14.14	109.28	85	1.1	2.5	100	0.11	0.75	83
Chilean recruits	133	11.18	69.35	87	ND	ND	ND	0.15	0.**	83
Lot IMS1569										
French volunteers	19	12.45	82.97	89	1.0	2.73	95	0.12	0.22	26§

NOTE. GMT, geometric mean titer; Pre, before vaccination; Post, after vaccination (see Subjects and Methods for times); ND, not done.

* Fourfold or greater rise in titer of antibody.

† Increase in titer of antibody ≥0.15 μg/ml.

‡ Increase in net OD ≥0.15.

§ $\chi^2 = 28.3$, $P < .0001$, compared with recipients of lot 53226.

Eleven (8%) of 136 Chilean recruits who received Vi antigen had temperatures >37.5 C; one had a temperature of 39.6 C with malaise, myalgias, headache, chills, and dizziness. Four (3%) recruits required bed rest in the 48 hr after vaccination. Vi more commonly produced local erythematous reactions ($P < .05$ vs. meningococcal vaccine, Fisher's exact test), with a larger area of erythema.

Table 1 also shows that no recipient of Vi lot IMS1569 had a temperature >37.6 C or any other systemic reaction.

Serological responses. Antibodies to Vi by passive HA. Paired serum specimens were available from 24 Maryland students, 133 Chilean recruits, and 19 French students. Table 2 shows the serological responses to the two Vi preparations. Eighty-five percent of the Maryland students and 87% of the Chilean recruits who received Vi lot 53226 had fourfold or greater rises in titer of antibody to Vi. A similar serological response was observed among recipients of Vi lot IMS1569.

Antibodies to Vi by RIA. Paired sera were available for RIA from 24 Maryland students and 19 French volunteers. The rate of seroconversions measured by RIA was high (95%–100%) among volunteers who received either Vi preparation (table 2).

O-specific antibodies. Vi lot 53226 elicited seroconversion in 20 (83%) of 24 Maryland students and in 111 (83%) of 133 Chilean recruits (table 2). Identical responses were observed with *S. enteriditis* LPS (data not shown). Vi lot IMS1569 elicited both lower rates of seroconversion and lower titers of O-specific antibodies. Only five (26%) of 19 volunteers had significant rises in *S. typhi* O-specific antibodies ($\chi^2 = 28.3$, $P < .0001$, for recipients of lot 53226 vs. lot IMS1569).

Discussion

In these clinical trials, Vi CPS lot 53226 (administered by jet-gun injection) produced fewer reactions than were reported for parenteral inactivated, whole-cell vaccine but more reactions than were reported for parenteral meningococcal vaccine or for oral Ty21a typhoid vaccine [1, 2]. These reactions—fever, malaise, and myalgias—were typical of those seen after inactivated, whole-cell vaccination. The number and severity of reactions probably render this preparation unacceptable for further clinical trials. Because different observers in a different country evaluated the reactions of volunteers to Vi lot IMS1569 and because the preparation was administered by syringe and needle instead of jet gun, we cannot fairly compare the reactions with those of volunteers who received Vi lot 53226. The jet gun may increase the rate and intensity of local and systemic reactions [13]. Nevertheless, the incidence of fever and other systemic reactions was less among recipients of lot IMS1569.

Vi lot 53226 contained sufficient LPS (5%) to induce significant rises in O-specific antibodies in most of the vaccinees. LPS is a pyrogen and elicits both local and systemic inflammatory reactions. The residual LPS was probably responsible for the fever, malaise, and local reactions. Vi lot IMS1569, however, had considerably lower levels of LPS (0.2%), induced a lower seroconversion rate of O antibodies, and produced fewer adverse reactions.

Reduction of LPS content to 0.2% in lot IMS1569 did not completely inhibit its immunogenicity; 26% of vaccinees responded with increases in titers of O-specific antibodies). Accordingly, immunization with Vi polysaccharide vaccine may interfere with the use of O-specific antibody assays for clinical and epidemiological studies. The use of Vi extracted from *C. freundii*, with its serologically different LPS, may circumvent this problem.

The rate of seroconversion after a single 50-μg injection of either Vi preparation was high, 85%–100%, depending on the Vi preparation and the assay for antibody to Vi (PHA or RIA). The immune response to Vi in the non-immune population (Maryland and French stu-

dents) was equivalent to that in the immune population (Chileans). In addition, the antibody responses to Vi elicited by lot 53226 with 5% LPS and by lot IMS1569 with 0.2% LPS were similar, which confirmed an earlier observation that LPS content of meningococcal polysaccharide vaccine did not affect the level of antibodies to CPS antigen [14].

The role of antibodies to Vi in providing immunity to typhoid fever in humans is unknown. In mice, immunization with Vi CPS confers a high degree of protection against challenge with *S. typhi* [15]. The significance of this is unclear, however, because mice are not natural hosts for *S. typhi* infections and do not develop a generalized infection resembling enteric fever. Protection against typhoid fever can be induced without antibodies to Vi, as shown by the experience with Ty21a vaccine, which lacks Vi antigen.

Thirty years ago, Hornick et al. carried out experimental challenge studies with *S. typhi* in volunteers previously immunized with a Vi vaccine prepared by Landy et al. [5]. This Vi was subjected to an acid treatment that removes all of the *O*-acetyl and part of the *N*-acetyl moieties and partially depolymerizes Vi polysaccharide [6]. The lack of these moieties may in part account for the poor protection provided by this preparation [6].

Vi lot IMS1569 induced high titers of antibodies to Vi in healthy volunteers and produced no adverse systemic reactions. The effects of age, nutrition, and chronic illnesses on production of antibodies to Vi after immunization are unknown. Further studies are needed to assess the role of serum antibodies to Vi in protection against typhoid fever and to demonstrate the efficacy of a Vi vaccine.

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